Fall 8-1992

An Evaluation of the Endocrine Response to Dystocia in the Primiparous Cow and Calf

Glenda M. Borcher

AN EVALUATION OF THE ENDOCRINE RESPONSE TO DYSTOCIA IN THE PRIMIPAROUS COW AND CALF

by

Glenda M. Borcher

A THESIS

Presented to the Faculty of
The Graduate College in the University of Nebraska
In Partial Fulfillment of Requirements
For the Degree of Master of Science

Major: Animal Science

Under the Supervision of Professor Edgar T. Clemens

Lincoln, Nebraska

August, 1992

AN EVALUATION OF THE ENDOCRINE RESPONSE TO DYSTOCIA IN THE PRIMIPAROUS COW AND CALF

Glenda M. Borcher, M.S.

University of Nebraska, 1992

Advisor: Edgar T. Clemens

Parturition is a very stressful event for both the cow and the calf. Endocrine changes occur which typically enable to calf to make the transition from a uterine to a gaseous environment. These endocrine changes are basic stress responses, necessary for an organism to maintain homeostasis. They include surges in catecholamine and cortisol secretion and changes in T_3 secretion in response to temperature changes.

It is well established that the stress response, if prolonged, can become detrimental. Prolonged stress can result in muscle wasting, immune impairment and gastrointestinal ulceration. If parturition is delayed or particularly stressful due to environmental factors, the endocrine stress response may become detrimental to the fetus and the neonate.

The catecholamine epinephrine is associated with hypertensive ulcers. Epinephrine stimulates the release of gastrin which stimulates the release of gastric acid in the

stomach. This study was set forth as an attempt to evaluate the level of stress associated with parturition and to identify the nature of the relationship between epinephrine, norepinephrine and gastrin.

In this study, epinephrine levels increased with the level of dystocia and an interaction between gastrin and epinephrine was identified in the calves at birth. As epinephrine levels increased over all concentrations of gastrin, there was an increase in calving score. This interaction resulted in the largest calving score at intermediate levels of gastrin (300 pg/ml) and calving score fell as gastrin levels increased.

However, the differences observed at birth were not apparent when the calves were 24 hours old.

The results of this study also suggest that there may be a relationship between the level of T_3 and dystocia. Calves with larger calving scores exhibited a lower T_3 secretion, even at 24 hours of age. This may indicate an impairment of the calves' ability to thermoregulate and may be an important factor in calf survival.

ACKNOWLEGEMENTS

I would like to express my deepest thanks to all of those individuals who assisted me in completing this project. First I would like to thank Dr. Edgar Clemens who served as both my major professor and friend. His patience and counsel were and still are very much appreciated.

I would also like to thank the members of my graduate committee: Dr. Rodney Moxley, Dr. Laverne Barrett, and Dr. Neal Woollen. Their comments and guidance in the completion of this project were invaluable. Thanks also to Deb Lambrecht for assisting me in the completion of my lab work and for spending many hours proofreading and editing and Suzy Foley for her excellent data entry work. Additional thanks go to Tammy Sorensen and the calving crew at Clay Center.

Thanks also go to Karla and Stacey, my two best friends who always believed that I really could get a thesis written and defended.

Finally, I thank my family for all of their support and love. Without their support, both emotional and financial, none of this ever would have been possible.

TABLE OF CONTENTS

Literature Review

Stress is considered a disorder resulting from exposure of an animal to a hostile environment (93). It can also be regarded as a part of the continuous attempt by animals to maintain themselves in a state of equilibrium with the environment (35). Although stress reactions are organized to protect the homeostatic state of the animal they may either enhance or diminish susceptibility to disease. In many instances, stress reactions themselves may induce pathologic change (8).

Since the turn of the century, largely as a result of the work of Cannon (12) and Selye (82), the physiological responses of the whole animal to hostile environments have been elucidated. However, both Cannon and Selye recognize that Claude Bernard's historical research laid the groundwork for their research (83, 13). Bernard was the first to develop the importance of the "milieu interieur" or internal environment of the body. In his article, Bernard stated "it is the fixity of the 'milieu interieur' which is the condition of free and independent life, all the vital mechanisms, however varied they may be, have only one object, that of preserving constant the conditions of life in the internal environment (13)." Cannon's work characterized the immediate response, the flight or fight syndrome, while Selye extended our understanding of the phenomena. In 1936, Selye (82) had

identified the stress response as being a "syndrome" that developed in three stages. Stage 1 developed 3-48 hours after the stressor. Observations include a rapid decrease in the size of the thymus, spleen, and lymph glands with a disappearance of fat tissue. Edema forms and there is a loss of muscular tone with the formation of acute erosions in the digestive tract. Selye refers to this as the "general alarm reaction" and indicates that these changes are very similar to those of histamine toxicosis or a surgical or anaphylactic shock. Stage 2 begins approximately 48 hours after the injury or stressor. The adrenals are greatly enlarged, the thyroid shows a tendency toward hyperplasia and general body growth ceases. During Stage 3, occurring after a period of 1-3 months, the organism loses its resistance and succumbs with symptoms similar to stage 1.

Since Selye and Cannon's early study, much work has been done to quantify stress and use less subjective measures of assessment (35). It is accepted that the adrenal gland plays a key role in regulating the response of an animal to stressors (35, 73). Breazile (8) indicated that an increase of glucocorticoid hormones develops in most distress cases. Stress stimulates the secretion of epinephrine from the adrenal medulla, norepinephrine from sympathetic nerves, corticosteroids from the adrenal cortex, and adrenocorticotrophin from the anterior pituitary (3). Each of

these responses, in turn, stimulates a cascade of events leading to the overall stress response.

ACTH. Adrenal cortical stimulating hormone (ACTH) is synthesized within the anterior pituitary. It is composed of 39 amino acids in all species (41). The primary target tissues of ACTH are the zona fasiculata and zona reticularis regions of the adrenal cortex. At these tissues, ACTH stimulates the synthesis and release of the glucocorticoids; primarily cortisol and corticosterone. In the bovine fetus, cortisol makes up 50-60% of the plasma corticosteroid one month prior to parturition. By 10 days prepartum, this percentage has increased to 90% of the circulating corticosteroid (49). Hunter, et. al., determined bovine fetal plasma levels of cortisol to be approximately 5 ng/ml 20 days prepartum with an increase to 74 ng/ml on the day of calving (49). Similar prepartum rises in cortisol levels have been seen 10-20 days prior to delivery in other species including the lamb, and goat (21, 86, 101).

The secreted ACTH stimulates the synthesis of corticosteroids in the adrenal cortex (3). At the adrenal cortex, ACTH activates the pathway converting cholesterol to cortisol. In the bovine, the predominant glucocorticoid is cortisol. An increase in glucocorticoid hormones contributes to a number of metabolic alterations, anti-inflammatory actions, immune competency modifications and development of

gastrointestinal ulceration (8). It is thought that glucocorticoids are produced in proportion to the intensity of the stress stimulus. Increased circulating levels of glucocorticoids have been used as an indicator of the intensity of distress responses in animals (8). ACTH concentrations have been shown to increase proportionally with increasing hypoxia (8). The increase in ACTH due to hypoxia would result in an increase in plasma glucocorticoids, namely cortisol. Work done by Keller-Wood, et. al., (60, 61, 62,) indicates that plasma levels of glucocorticoids are set by stress intensity and regulated by close controls. This leads to the conclusion that circulating levels of glucocorticoids can be used as a non-subjective indicator of the intensity of stress response in animals (8).

Cortisol. Cortisol is a C-21 steroid having a molecular weight of approximately 363 g/mol (41). Typical bovine plasma concentrations range from 5 ng/ml to 200 ng/ml (32). The major physiological role of cortisol is to increase carbohydrate metabolism and sympathetic nerve function (41). Cortisol acts to enhance the lipolytic actions of catecholamines on adipose tissue. The free fatty acids released from fat cells serve as metabolic substrates necessary for survival. Prolonged hypercortisolism eventually leads to muscle wasting, hyperglycemia, atrophy of the immune

system, vascular derangements, and gastrointestinal ulceration (41).

Jones, et. al. reported that cortisol is a lipid soluble molecule (56). Because of this lipid solubility, cortisol is able to pass the placental barrier freely. This influx of maternal cortisol, may lead to hypercortisolism in situations of prolonged calving stress. Additionally, in work conducted by Jones, et. al (58) using pregnant ewes that had chronically implanted vascular catheters, it was seen that physiological rises in maternal plasma catecholamines, resulting from epinephrine infusion, resulted in a rise in fetal plasma ACTH. This lead Jones and coworkers to conclude that physiological rises in maternal plasma catecholamines during dystocial delivery may also stimulate that release of ACTH from the fetal pituitary which may also contribute to hypercortisolism.

Glucocorticoids act to regulate catecholamine biosynthesis in the adrenal medulla (3). Thus maternally induced hypercortisolism within the fetus may result in modified levels of fetal plasma catecholamines. Graham, et. al., (37) showed that cortisol caused epinephrine release in fetal adrenal cells but had no effect on the release of norepinephrine or dopamine. These results are consistent with work utilizing fetal ovine adrenomedullary cells. Cheung (16) reported that cortisol selectively stimulates the release of

epinephrine without affecting norepinephrine or dopamine. Cheung (16) indicated that this increase in epinephrine secretion was a consequence of cortisol increasing the enzyme activity of PNMT (phenylethanolamine N-methyltransferase) in the adrenal medulla. Similar conclusions have been drawn by other authors (74, 55, 72, 103). PNMT is the enzyme responsible for the methylation that converts norepinephrine to epinephrine (41).

Catecholamines. The primary catecholamines are epinephrine, norepinephrine, and dopamine. Of these, epinephrine was the first to be characterized. The true structure of epinephrine was determined by Aldrich in 1901 (41). Epinephrine has since been determined to be the main hormone produced by the chromaffin tissue of the adrenal medulla (3, 41, 92, 103). Norepinephrine was discovered much later (41) and is secreted from the sympathetic nerve endings as well as from the adrenal medulla. For this reason, norepinephrine is viewed as being a classic example of a neurotransmitter.

Both epinephrine and norepinephrine are considered to be phenolic amines. However, norepinephrine is a primary amine whereas epinephrine is an N-methylated secondary amine (41). The pathway of catecholamine synthesis begins with the amino acid phenylalanine and through a series of hydroxylation, decarboxylation, and methylations results in the

catecholamines (41). The cathecholamine synthesis pathway is outlined below (41).

The amino acid phenylalanine is hydroxylated to form tyrosine. This reaction is catalyzed by the enzyme phenylalanine hydroxylase. Tyrosine is converted to dihydroxyphenylalanine (DOPA) by the enzyme tyrosine hydroxylase. DOPA is then decarboxylated to dopamine by DOPA decarboxylase. Dopamine is then hydroxylated by dopamine beta-hydroxylase to form norepinephrine. Conversion of norepinephrine to epinephrine is caused by PNMT, an enzyme found only in epinephrine synthesizing cells (41).

In response to stress, there is an increase in the activity of the sympathetic nervous system and the adrenal medulla resulting in the discharge of epinephrine and norepinephrine into the blood stream (3). They cause increases in heart rate and blood pressure, peripheral vasoconstriction, and mobilization of the liver energy store, glycogen (35). Additionally, the catecholamines act to dilate the bronchi to facilitate pulmonary function (92). These responses to catecholamine release act to prepare the body for the "fight or flight" response. In calves and lambs, Richet and co-workers reported that catecholamines play a major role in a variety of neonatal adaptations (77). These include non-shivering thermogenesis and lung maturation. Results of work by Padbury, et. al also suggest that the cardiovascular,

metabolic, thermogenic and endocrine changes that occur during the transition to extrauterine life, occur, at least in part, in response to increases in plasma catecholamines (71).

In most mammals, the main catecholamine produced is epinephrine (3). While this is true of the adult animal, the primary fetal catecholamine is norepinephrine (30, 54, 51, 64). However, as gestational age increases, the proportion of epinephrine increases (51, 55, 86).

The proportion of epinephrine to norepinephrine changes with respect to the source of stress. Much research has indicated that physical exercise causes the release of primarily norepinephrine while epinephrine remains low or variable (4, 17, 24, 27, 31, 34, 38, 63). Emotional stress appears to cause a dramatic increase in epinephrine but not norepinephrine (27, 34). In mature wethers, cold exposure has been shown to cause a rapid and extensive increase in plasma norepinephrine and epinephrine (98). Thompson, et al. (98), reported norepinephrine concentrations of .24 ng/ml prior to cold exposure and 1.11 ng/ml after cold exposure. Similarly plasma epinephrine increased from 0.07 to 0.25 ng/ml. Comparable responses to acute cold have been reported in humans (53), laboratory rats (18), nonlactating dairy cows (1), and steers (78). Thompson also observed that entrance of laboratory personnel into the experimental chamber caused a dramatic increase in the wethers' catecholamine concentrations

(norepinephrine 0.29 to 1.04 ng/ml and epinephrine 0.07 to 0.40 ng/ml) in a ten minute period (98).

T_3. An additional response of an animal to cold stress is an increase in T_3 secretion. T_3 (triiodothyronine) is a phenolic amine synthesized in the follicles of the thyroid gland. The primary effect of T_3 is to cause thermogenesis. At parturition, increased levels of cortisol affect thyroid function of the neonate (70). Nathanielsz and Fisher report that infusion of cortisol into fetal sheep results in lowered T_4 levels and increased T_3 levels. As these changes occurred within 24 hours of infusion, it was concluded that the changes are likely to be caused by cortisol itself rather than being secondary effects of other hormonal changes that occur as a result of cortisol induced premature delivery (39).

It was also reported by the same authors that immediately after birth, there is a marked increase in neonatal plasma T_3 levels (70). This increase may result from cortisol of either maternal or fetal origin. This resulting increase in fetal T_3 is thought to play a thermogenic role in the first hours postpartum (42). Cold stressed Brahman calves responded with increased serum thyroid concentration, indicating that the cold Brahman calves were attempting to increase their metabolic rate so as to increase thermogenesis (36).

Hematology. Stress induces changes in the cellular components of blood. These changes are primarily due to the

effects of the glucocorticoids. The total and differential counts of newborn calves, as compared to the normal adult, exhibited leukocytosis, neutrophilia, eosinopenia, and relative lymphocytopenia (29). These are all conditions characteristic of the leukocyte response to ACTH. As a result of ACTH release, glucocorticoids are released from the adrenal cortex, thus the leukocyte response to ACTH is probably mediated through the glucocorticoids. Eberhart (29) reported that this leukocyte pattern is suggestive of glucocorticoid-mediated effects. Tennant, et. al (97), reported that calves exhibited a mean neutrophil:lymphocyte ratio which was 2.8 at birth. Similar results were reported by Eberhart (29). Typically within the first week of life the number of neutrophils fell and the number of lymphocytes increase to levels similar to that of the adult animal (97, 29). According to Eberhart the increased normality of the leukocyte pattern became apparent as cortisol decreased (29).

Eberhart (29) suggests that the lymphopenia associated with stress may have immunological ramifications. In the newborn calf, it has been proposed that the increased susceptibility to bacterial infection may be enhanced by high plasma cortisol concentrations at birth and their effects upon the lymphocytes (29).

Stress of Parturition

Stress is not limited to environmental factors. Stress can originate from sources within the individual including physiological/biochemical (i.e. hypoglycemia, prolonged exercise, hypoxia, injury and other trauma) or psychological/psychosocial (i.e. behavioral and emotional responses) (2). The process of parturition is generally well accepted as being stressful. The stress is felt by both the mother and the fetus. In 1935, Canon first described how the fetus is surrounded by a warm fluid matrix (14). This warm fluid matrix is very similar to the fetus's own internal environment. However, at the time of parturition, the environment changes from warm fluid to one far different from the neonate's internal environment, cold and gaseous.

In uncomplicated parturition, the stress reactions are superimposed upon the normal endocrine and neural mechanisms demanded for extrauterine life (9). The newborn is prepared, in part, through increased adrenal secretion of cortisol. There is a significant increase in fetal plasma cortisol due to a signal originating within the fetal hypothalamic-pituitary axis. In addition to cortisol originating from the fetal hypothalamic-pituitary axis, it has been shown in goats (102) and ewes (28) that cortisol can partly cross the placenta, from mother to fetus. Regardless of source, this increase in cortisol has been seen in sheep, goats, and cattle

(49, 102). Within the neonate, cortisol induces normal pulmonary, intestinal, and brain development, pulmonary surfactant production and the preparation of liver glycogen for use as energy upon cessation of placental blood flow. In fetal lambs, plasma cortisol concentrations begin to increase significantly 10-15 days before parturition (100). Eberhart, et. al, (29) reported plasma cortisol concentration of 12.1 ug/dl in the newborn calf and 5.0 ug/dl in the dam. Neonatal plasma cortisol levels dropped to 4.9 ug/dl at 12 hours of age. Stott and Reinhard (95) report maternal plasma cortisol values at parturition of 1.92 ug/dl (19.2 ng/ml) while Comline, et. al (21) report values ranging from .5-2.0 ug/dl (5-20 ng/ml). Comline and co-workers (21) results showed no consistent changes in maternal plasma cortisol as gestation proceeded and no significant rise before and during parturition.

In the calf at parturition, plasma cortisol values range from 9.4 ug/dl (52, 21) to 12.3 ug/dl (95). It appears that the value is dependent upon the level of parturition stress experienced by the animal. Concentrations of plasma cortisol are higher in dystocial calves as compared to eutocial calves (52) which suggests that the dystocial calves undergo a greater stress response. The relationship between plasma cortisol and the difficulty of the delivery was reported by Massip (68). Cortisol concentrations in normal calves was

68.21 ng/ml whereas for dystocial calves the concentration was 81.25 ng/ml. Although these values were not determined to be statistically different, the author suggested a strong correlation between the nature of the birth and the level of plasma cortisol (68).

Fetal blood pH and blood gases remain stable during gestation (22). During parturition, labor contractions produce transient periods of hypoxia but normally no changes are observed in umbilical blood (76) unless abdominal straining occurs. At parturition, interference with placental blood flow and gas exchange can result in the elevation of PCO_2 producing respiratory acidosis (9). In the eutocial situation, this acidosis is transient in the calf due to compensatory mechanisms (96).

The fetus is well equipped to withstand the stress of normal delivery (65). At parturition there is a surge of hormones that prepare the neonate to survive outside the womb (65). During parturition, the fetus produces high levels of catecholamines. In the adult the primary secreted catecholamine is epinephrine whereas in the fetus, norepinephrine is predominantly secreted (65). Catecholamine secretion at birth may influence pulmonary adaptation to extrauterine life (72). Additionally, the secretion of catecholamines afford the fetus much protection from hypoxia (65). Lagercrantz and Slotkin (65) demonstrated this in

neonatal rats. When the adrenals were intact, the rats secreted large amounts of catecholamines and were able to survive intense and prolonged hypoxia. When catecholamine action was blocked, the ability to survive hypoxia was lost. In humans, normal birth processes have been shown to yield an increase in plasma catecholamines equivalent to five times the catecholamine concentration in the resting adult (65). In sheep, maternal epinephrine levels increase three-fold during the last 30 minutes of spontaneous delivery as compared to an eight-fold increase in fetal epinephrine levels during the same time period (55).

Eliot and colleagues indicate that wide fluctuations in the concentrations of maternal catecholamine reflect the influence of environmental factors as well as the physiological stress associated with parturition (30). In work done by Gu and Jones (39) maternal infusion of epinephrine in sheep resulted in an immediate rise in fetal norepinephrine to a plateau level. Fetal norepinephrine increased 2-fold during the maternal infusion of epinephrine. This probably occurred due to the decreased uterine blood flow resulting from the maternal epinephrine. As uterine blood flow decreased the placenta became hypoxic. A rise in fetal epinephrine was not detected until 60 minutes of infusion had elapsed (39). The immediate increase in norepinephrine and later increase in epinephrine suggest that norepinephrine is

the predominant fetal response to hypoxia. However, if the stress of hypoxia is prolonged epinephrine is released into fetal circulation (39). This may also be the case for other stress situations that cause the release of epinephrine into maternal circulation.

Hypoxia induces a large increase in the level of catecholamine secretion from the adrenal medulla (57). In humans, moderate levels of hypoxia, as determined using blood gas measurements, induce a 10 fold increase in plasma catecholamine concentrations. Holden, et al., (46) quantified arterial catecholamine levels to be 9.5 nm/liter and 252 nm/liter in normal and asphyxiated infants at delivery respectively. As early as 1926, it was determined by Houssay and Molinelli (48) that asphyxia produces a marked discharge of epinephrine (98). In fetal lambs, hypoxia resulted in increases of both epinephrine and norepinephrine (20). The catecholamine levels associated with mild hypoxia in fetal sheep have resulted in large cardiovascular effects (80). The results have been confirmed in the fetal lamb by Comline and Silver (23) and in the fetal calf by Jones and Robinson (57).

In the adult negative effects of increased adrenal activity include impaired hair growth, reduced reproductive capacity and suppressed immunologic function (44) which can result in the occurrence of diseases of adaptation (44). It is not unreasonable to expect neonates to respond to stress in

a similar manner. Research has suggested that dystocial calves respond to stress with hyperadrenalemia (95) and that this intensified adrenal activity increases the mortality rate due to extended parturition (94).

Dystocial births can result in an increase in the level of hypoxia experienced by the calf. Under anaerobic conditions the percentage of norepinephrine secreted is reduced and the balance of the catecholamine output is largely maintained by epinephrine. This may result from the mechanism of catecholamine secretion. It appears that norepinephrine secreting cells are more dependent upon oxidative metabolism for their secretory activity than epinephrine containing cells (81).

The severity of hypoxia experienced by the fetus also directly impacts the level of acidosis during parturition. Blood pH and P_{CO2} have been shown to be significantly higher in calves after a difficult delivery than normal delivery (68). Similar observations have been made on the human infant, the piglet, and the foal (106). Massip (68) reported that in addition to the significantly lower blood pH the dystocial calves had a higher plasma cortisol concentration than normal calves. However, these difference were not statistically significant. Massip concluded that "the values of acid-base parameter are a reflection of the disturbance of the normal values, brought about by the labour and delivery and that the

adrenal gland reacts to this disturbance by secretion of cortisol (68).

Additionally, anxiety stress or fear prolongs the act of parturition in several species through a decrease in myometrial contractility induced by a release of epinephrine (42). Initially, the myometrium will contract in response to epinephrine, a response mediated by the alpha-adrenoreceptors. However, simultaneous stimulation of the beta-receptors causes inhibition of myometrial contractility (41). This prolongation of parturition may cause a prolonging of the stress upon the fetus with ramifications as outlined above.

Causes of Dystocial Parturition

There is evidence from several sources that maternal stress may be deleterious to the fetus. Woodward and Clark (107) recognized that dystocia was a significant factor in the mortality of the neonatal calf. Since this time, a large amount of research has been devoted to elucidating the causes of dystocia. Many factors contribute to the incidence of dystocia including malpresentation, which can be a problem regardless of the age of the dam and affects all parities equally, and factors such as calf size and dam size, which have a greater effect at the first parity (99).

Dam-related factors contributing to the incidence of

dystocia have been shown to include pelvic diameter, hip width, rump length, and body weight (5, 66, 11). A correlation exists between the age of the dam at calving and the incidence of dystocia. Frequency of dystocia tended to decline as dams increased in age (10, 66, 11). It is thought that the source of decreasing dystocia is due to smaller pelvic area in younger dams and an increased body weight in the older dams (66, 11). The incidence of dystocia has also been associated with the breed of the dam and of the sire. Hereford cows have more calving difficulty than Angus cows (66). Calves sired by Charolais, Simmental, Limousin and South Devon bulls experience more calving difficulty than those sired by Hereford, Angus and Jersey bulls (66).

Calf-related dystocia factors include the size and sex of the calf. Larger calves have a greater mortality, particularly at first parity (99). Calving difficulty increased approximately 2.3% for each kilogram increase in the birth weight of the calf (66). Dystocia is more pronounced in male calf births than female calf births (66). In research done by Brinks, et. al. (10), dystocia occurred in 10.5% of the male births but only 7.1% of the female births. The increased incidence of dystocia with male births may be due to the higher birth weight of male calves. Laster's (66) work showed that male calves tended to be heavier at birth than the female calves. Males calves averaged a birth weight of 35.12

kg as compared to 32.10 kg for the female calves.

Negative Effects of Dystocia. According to Stott (94) it is believed that higher mortality in dystocial calves is related to the stress from delayed and difficult parturition. Negative effects resulting from maternal stress may include increased incidence of premature labor, stillbirth, and growth retardation (79). Along with increased mortality, neonatal weakness may be a major result of dystocia. Neonatal weakness is the major cause of low calf viability during the first 24 hours of life (25). According to Dennis (25) and Kasari (59) many factors contribute to neonatal weakness in calves.

Weakness factors include: 1) fetal stress due to hypoxia; 2) birth trauma (including meningeal hemorrhages, fractured ribs and localized edema of head and/or hind limbs); 3) congenital malformations, prematurity, dysmaturity; 4) intrauterine infections; 5) placental dysfunction (causing underweight and underdeveloped calves); 6) extremes in birth weight; 7)hypothermia; 8) incidence of twins; 9) postnatal infection; and 10) starvation.

RATIONALE FOR PROJECT

Catecholamine-Gastrin Interaction

The gastro-intestinal hormone gastrin was isolated in 1964. In most mammals, gastrin circulates in at least 3 molecular forms: Gastrin-34 (Big Gastrin), Gastrin-17 (Little Gastrin), and Gastrin-14 (Mini-Gastrin) (41). Big-Big gastrin has been found in tissues and circulation but has not been characterized chemically or biologically. It is assumed to have a molecular weight greater than that of Gastrin-34 as a result of elution from Sephadex gel-filtration columns. Gastrin hectadecapeptides have been purified from the antral mucosa of several species, including man, pigs, dogs, cats, sheep, and cattle. The gastrins have been found to differ by only 1 or 2 amino acid substitutions in the middle of the linear peptide chain (104).

The primary effect of gastrin is to increase gastric acid (primarily hydrochloric acid) secretion, promote gastrointestinal growth, and antral motility (41). Food is the primary stimulus for gastrin release. However, epinephrine in low concentrations will stimulate gastrin release and results in higher acidity of gastric juice and acid output (47, 90, 19). Christensen and Stadil (19) displayed that in humans, a low dose of epinephrine (5 ng/kg/minute via intravenous infusion) stimulated gastrin

release and acid secretion, but the higher dose (50 ng/kg/minute via intravenous infusion) while stimulating gastrin release, did not change acid secretion. In humans, Stadil and Rehfeld (90) showed that serum gastrin concentrations increased immediately upon administration of epinephrine, but returned to basal levels within 30 minutes. In dogs, the reported half-lives of gastrin are as follows: 3 minutes for Gastrin-17, 9 minutes for Gastrin-34 and 90 minutes for Big-Big Gastrin (104).

In comparison, norepinephrine in the same study caused a very small increase in gastrin release at the low dose (5 ng/kg/minute) with no change in acid secretion. At the high dose (50 ng/kg/minute) norepinephrine inhibited acid secretion without any change in gastrin concentration (19). It is unknown why these hormones appear to exert opposite effects. It has been proposed that epinephrine stimulates beta-adrenergic (stimulatory) receptors while norepinephrine stimulates alpha-adrenergic (inhibitory) receptors (90, 19).

As early as 1972, Hayes, et. al. (45) suggested that stimulation of gastrin release by catecholamines may link stress with peptic ulcerations. In the fetus, the primary catecholamine has been shown to be norepinephrine (30, 39, 33, 55, 85). During prolonged parturition, epinephrine levels begin to rise within fetal circulation (30, 39).

This rise in fetal epinephrine may be of fetal origin or

may be of maternal origin. There is some argument as to whether or not catecholamines transverse the placental barrier. According to Richet, et al., (77) catecholamines do not cross the placenta easily in humans, ovines, or other species. However, Lagercrantz and Bistoletti (64) report that catecholamines pass the placenta easily but the influence of maternal catecholamines on the fetus is negligible due to the high monoamine-oxidase activity within the placenta and the rapidity of catecholamine metabolism. It has been reported that the monoamine-oxidase and catechol-o-methyltransferase activity of the placenta acts to defend the fetus from a damaging excess of catecholamines from maternal circulation (69). However, in research conducted by Wei Gu and Jones (39) it was proposed that although there is an effective placental permeability barrier to epinephrine it may not be maintained over extended periods of time. In this work, 0.5 ug/minute/kg of body weight of epinephrine were infused into the circulation of pregnant ewes. Over a 60 minute infusion period fetal epinephrine had risen at least 10-fold (39).

Thus, it is possible that gastrin levels in the fetal calf are not affected until dystocia is of sufficient severity to cause an increase in fetal epinephrine levels. Since the primary fetal catecholamine is norepinephrine, the dystocia may have to be of long enough duration to cause a breakdown in the placental catecholamine barrier allowing the influx of

maternal epinephrine into fetal circulation and the resultant effects on gastrin secretion.

Calf Perinatal Maladjustment

History. Weak calf syndrome (WCS) has been known by a variety of names including neonatal idiopathy polyarthritis, Ward's syndrome, Bitterroot Crude, EBA (empty bank account) and other descriptive terms (26). This condition was first recognized in the Bitterroot Valley of Montana in 1964. The morbidity rate varied from 5 - 15% and the case mortality rate was often as high as 80% (7, 15). In 1968, approximately 400 cases of the syndrome were diagnosed in the Salmon-Challis area, and in 1973 the number of reported and treated cases reached a thousand (15). It is estimated that the 1973 outbreak resulted in a loss of $131,000. However, it is difficult to estimate total losses to the livestock industry. Calves who have recovered from WCS exhibit unthriftiness and poor growth (15). Since the 1970's, cases of WCS have been reported in Utah, Oregon, Colorado, Nevada, Nebraska, Washington, Wyoming, Iowa, and Alberta, Canada. (15, 50, 91). More recently, WCS has been reported in Northern Ireland, the Netherlands, and England (88).

Clinical Signs and Postmortem Lesions. Calves affected by WCS exhibit a number of clinical signs. These include subcutaneous edema (91, 87, 7, 105, 26) and hemorrhages of the

carpal and tarsal joints (91, 26, 7). As a result of painful joints, the calves are often reluctant to stand. According to Ward (105) 80% of all WCS affected calves exhibit blood-tinged synovial fluid in the tarsal and carpal joints. Additionally, at necropsy, prominent lesions include erosion or ulceration of the gastrointestinal tract (26, 7). Ulcerative lesions of the forestomach have been seen in approximately 30% of reported WCS cases (26). Other clinical signs include petechial and diffuse hemorrhages on the conjunctiva and third eyelid (15, 26), edema of the gall bladder and bile duct (105) and reduced thymus mass (26, 105, 7). Also, histopathologic lesions have been found that indicate a mild to severe depletion of the cell mediated immune system (105).

Etiology. WCS has been observed most frequently during inclement weather conditions (50, 7). The stress of inclement weather has been alluded to by both cattlemen and practicing veterinarians as a causative factor in WCS (15) while others consider it to be only a factor that aggravates the already present WCS condition (91). However, WCS losses have been considerably higher during severely cold seasonal temperature as often associated with heavy snowfalls or storms (26).

In addition to being more frequently seen during inclement weather, WCS is also more frequently seen with first-calf heifers (91, 7). First calf heifer or recently introduced herd replacements more commonly give birth to

calves affected by WCS at birth or shortly thereafter (7). Records and herd histories have indicated that a cow delivering a WCS affected calf will rarely have another affected calf (7, 105, 91). However, in Northern Ireland, it is reported that many of the weak calves are delivered from multiparous animals (88).

To date, no definitive cause of WCS has been found. According to Ivanoff and Renshaw (50) "The prevailing view has been that WCS is of infectious origin because of the pattern of susceptibility." Bacterial and/or viral causes have thus been investigated. Bovine viral diarrhea virus has been most frequently isolated from WCS affected calves (67) but has not been recovered from the majority of WCS calves (15, 67). Other isolated infective agents include Haemophilus somnus (87, 91, 26), adenovirus types 2, 5, and 7 (105, 26), IBR, brucellosis, leptospirosis and anaplasmosis (26). Haemophilus somnus has been shown to be able to reproduce the polyarthritis associated with WCS but not the other associated lesions (26, 91). BVD and adenovirus have reproduced subcutaneous hemorrhagic lesions similar to those seen with WCS but failed to produce lesions of the internal organs such as the kidneys, adrenal glands, and liver (26). It has been suggested that if WCS is bacterial or viral in origin, it may be one or more agents acting in a synergistic relationship (105). The aforementioned infective agents have been shown to

accentuate a WCS problem in addition to their own primary disease problems but have not been shown to be causative agents of WCS.

A dietary cause has also been proposed for WCS. A direct correlation has been shown between the incidence of WCS and the level of crude protein intake per cow per day during the last 60 days of gestation. Dierks (26) found that when the cows were on a diet of greater than 2 pounds of crude protein per day, the incidence of WCS was very low. Stauber (91) reported that for each 0.045 kg (0.1 lb) of crude protein consumption below a minimum of 0.9 kg (2 lb) of crude protein/pregnant animal/day there was a 1% increase in WCS in 12 herds under field conditions.

There is some thought that fetal hypoxia may be an important cause of WCS. Blood, et al., (7) reported that a condition similar to WCS was produced experimentally by clamping the umbilical cord of the fetus in utero for 6-8 minutes followed by a Caesarean section 30-40 minutes later. Calves born following the procedure did not make the usual effort to sit up in sternal recumbency and were unable to stand even when assisted. The calves were dull and inactive and their sucking reflex was poor or absent. The calves died 10-15 minutes after birth or survived for only up to 2 days. A similar condition is seen in foals that have experienced fetal and neonatal hypoxia (7). These foals, called "barkers"

and "wanderers" (also known as convulsive and dummy syndrome) are of low birth weight, weak, unable to stand and do not display normal suckling reflexes. These responses, as seen in the "barker" foals and the hypoxic calves are consistent with irreparable brain damage (7).

The brain is one of the highest oxygen consuming tissues of the body and as a result is one of the tissues most rapidly and dramatically affected by hypoxia (89). In humans, as a result of severe hypoxia, the respiratory center of the brain often becomes depressed as a result of its own oxygen deficit. This depression results in a decrease in respiration until it actually stops, in spite of the stimulatory effect of the chemoreceptors on respiration during hypoxia (40). In adult humans, hypoxia results in decreased ability to perform discrete motor movements. This may be similiar to the inability of the hypoxic calves and foals (7) to stand and display normal suckling reflexes.

Hemorrhagic lesions are a common finding in infants who die following hypoxic births. These lesions include small hemorrhages on the thymus, lungs, and heart; massive hemorrhages from the liver, spleen and into the muscles as well as cranial hemorrhages (6). These hemorrhagic lesions could potentially be related to those associated with weak calves.

Fetal hypoxia may result from dystocial births (81). As

outlined above, calf size and malpresentation are significant factors contributing to dystocia. However, Dierks reported that in most cases of WCS the calves are in a normal position and are of a size such that normal delivery should proceed uneventfully. However, the calves observed by Dierks were not delivered without assistance but very little effort was exerted by the herdsman in delivering these calves (26). Ward reported that cows delivering weak calves exhibited apparent uterine inertia (105). It may be that stress resulting from the environment or handling prior to parturition results in epinephrine release with the subsequent decline in myometrial contractility as described earlier. It may be this prolonging of parturition that contributes to the increase in fetal hypoxia and the stress experienced by the calf rather than the actual mechanics of parturition.

WCS may be more correctly classified as a problem rather than a disease due to its possible multiple etiology. Smyth, et. al (88) concluded that the condition termed weak calf syndrome may in fact be the result of more than one disease process. Several pathogenic agents have been isolated from WCS calves but researchers have been unable to establish a causal relationship. Additionally, inclement weather and poor nutrition have been proposed as causes. One common theme associated with all of these is that they put the cow and the fetal or neonatal animal under stress. Thus in this project

we attempted to examine the effects of stress, resulting from calving difficulty, upon the neonatal calf.

Material and Methods

A total of 112 MARC-MARC III, two-year-old, first-calf heifers from the South Central Research and Extension Center near Clay Center, Nebraska were utilized in this project. MARC III refers to a composite breed of cattle that is 1/4 Red Polled, 1/4 Angus, 1/4 Hereford, and 1/4 Pinzgauer. MARC-MARC III refers to an animal produced as a result of mating a MARC III cow and a MARC III bull.

During the 1990 calving season, 52 heifers were sampled and in 1991, 60 heifers were sampled. Each year, herd size consisted of approximately 120 heifers. The cattle were kept in a calving pasture with free access to water and were fed corn silage once a day at a rate of 50 pounds of corn silage per head.

Procedure. Calving for this study occurred between March 5-May 1, 1990 and March 14-April 26, 1991. The heifers were checked at one hour intervals for signs of calving. After having exhibited Stage II labor for one hour, heifers were brought into the calving barn and placed in a calving stanchion. Stage II labor was consists of rupture of the allantochorion and escape of fluid from the vulva and abdominal straining. If a malpresentation was evident, the heifer was brought into the calving barn immediately. An experienced herdsman assisted each heifer and assessed the level of calving difficulty. The MARC calving score assigned to each parturition was based on the following criteria:

Sas Score	MARC Score
1	1 = Denotes an unassisted delivery
1	2 = Little Difficulty. Assistance given by hand but no jack or puller used; assistance actually may not have been required.
2	3 = Little difficulty with calf jack. Assistance given with jack or puller. Little difficulty was experienced; may have actually been able to pull by hand.
2	4 = Slight difficulty. Assistance given with jack or calf puller. Some difficulty was encountered even with pullers being used. Difficulty was not enough to cause death or damage to cow or calf. Typically pulling duration is of less than 30 minutes.
2	5 = Moderate difficulty. Calf jack was used; difficulty was such that personnel had fairly difficult task. Minor damage may have been done to cows or damage to some calves. Typically pulling duration is of more than 30 minutes.
3	6 = Major difficulty. Calf jack used and major difficulty encountered. Caesarean section probably should have been called for in some instances, for example, a severe hiplock with a delivery of longer than 30 minutes.
Caesarean Section	7 = A caesarean section.
Malpresenation	8 = Abnormal presentation or posture. Posterior, head back, leg back, etc. Presentation was recorded; calf delivered with assistance.

For the purposes of statistical analysis, the calving scores were regrouped according to the SAS Score. This regrouping was utilized to minimize subjective differences in MARC

calving scores due to variation between herdsmen. All scores presented in the results section are SAS scores.

A calving score of MARC Score of 1 was assigned to heifers delivering calves unassisted and those heifers that would have delivered unassisted calves if they had not been brought into the calving barn after 1 hour of labor. Calves that were presented abnormally were received two scores: an 8 indicating the malpresentation and a score of 1-7 indicating the difficulty encountered during the delivery. In 1990 and 1991 the number of cow/calf pairs sampled per level of calving difficulty was as follows:

Level of Difficulty		# of cow/calf pairs	
		1990	1991
Sas Score	MARC Score		
1	1	10	26
1	2	3	2
2	3	16	19
2	4	14	7
2	5	7	2
3	6	1	3
C-Section	7	1	1
Malpresentation	8	5	3

In 1990, three MARC calving scores of 2, two MARC scores of 3 and two MARC scores of 5 were also malpresentations and received the additional score of 8. In 1991, one MARC score of 3 and two MARC scores of 6 were also malpresentations. These were denoted by the notation "8/2" or "8/6."

Dates and times of calving and blood sampling were recorded. Additionally, at birth, information regarding the duration of the pull, drugs administered to the calf or

heifer, weather, and any sampling or animal handling problems were noted. Upon the arrival of the calf, care was taken to ensure that spontaneous respiration began. If deemed necessary, calves were administered oxygen to facilitate respiration. The heifer-calf pair was placed in a stall with fresh straw and left undisturbed. The pair was periodically checked to evaluate the calf's progress with regard to standing and suckling. Approximately 24 hours postpartum the heifer and calf were released into a post-calving pasture containing other cow-calf pairs. At 24 hours, information regarding the cow-calf interaction, health of the heifer and the calf, and blood sampling problems were recorded.

Blood Sampling. Immediately following parturition, three blood samples were drawn from the heifer and calf via jugular venipuncture[1] using an 18 g needle on the calves and a 16 g needle on the heifers. Blood was drawn into a 25 ml Sarstedt[2] serum collection tube and a 10 ml Sarstedt plasma collection tube using heparin at a concentration of 12.5-12 I.U./ml blood as the anticoagulant. An additional sample was drawn and aliquoted into a vacutainer containing ethylene glycol- bis(β-aminoethyl ether) N, N'-tetraacetic acid (EGTA) and reduced glutathione[3]. This procedure was followed when sampling both

---

[1] 18 g 1 1/2" and 16 g 1 1/2" Color-Klear Hub Needles, Becton-Dickinson, Becton-Dickinson Division, Rutherford, N.J.

[2] Sarstedt, 3586 Arden Rd., Hayward, CA 94545

[3] Special vacutainers, 13 x 75 mm, 5 ml, Amersham Corp., Arlington Heights, IL.

the cow and the calf. The calf was again sampled in the same manner at 24 hours of age. In the cases of caesarean birth, blood samples were also taken via jugular venipuncture on the heifer and the calf. The heifer sample was taken upon extraction of the calf.

Blood Sample Analysis. Blood smears were made for each animal immediately following sampling from the 10 ml Sarstedt plasma collection tube. Blood smears were stained using Leuko-Stat[4], a water-soluble modification of Wright's stain, for determination of differential blood cell counts. All collection tubes (10 ml Sarstedt, 25 ml Sarstedt, and EGTA/glutathione vacutainer) were centrifuged at 2300 x g for 20 minutes[5]. Plasma was harvested and placed in plastic tubes[6], labelled and frozen at -20 C for future analysis. Serum collected from the 25 ml Sarstedt tube was handled in the same manner. Plasma from the 10 ml Sarstedt tube was analyzed for T_3[7] and cortisol[8] while the serum collected in

---

[4] Leuko-Stat, Fisher Diagnostics, Orangeburg, NY 10962.

[5] Beckman Model TJ-6 Centrifuge, Instruments, Inc., Palo Alto, CA 94304.

[6] Plastic Scintillation Vials, Wheaton Scientific, Millville, N.J.

[7] T_3 Kit, Diagnostic Product Corporation, Los Angeles, CA. 1988.

[8] Cortisol Kit, Diagnostic Products Corporation, Los Angeles, CA. 1986.

the 25 ml Sarstedt tube was analyzed for gastrin[9]. These analyses were carried out using an automatic gamma radiation counter[10]. Plasma from the glutathione tubes was analyzed for norepinephrine and epinephrine concentrations using high performance liquid chromatography[11].

Radioimmunoassay (RIA) Methodology. The T_3 and cortisol concentrations were determined using a solid-phase RIA. In both assays, ^{125}I-labeled hormone competed with sample hormone for antibody sites. Polypropylene tubes were coated with antibody so decanting the supernatant sufficed to terminate the competition and isolated the antibody-bound fracton of the radiolabeled hormone. Counting the tube in a gamma counter yielded a number which was converted to hormone concentration. Additionally, in the T_3 RIA, the reaction took place in the presence of blocking agents which served to liberate carrier protein-bound T_3, allowing the assay to determine total T_3.

The gastrin RIA was a double-antibody ^{125}I assay. Isotope labeled gastrin competed with sample gastrin for sites on gastrin specific antibody. Following incubation, separation of bound hormone from free was achieved by the PEG (polyethylene glycol)-accelerated double antibody method.

---

[9] Gastrin Kit, Diagnostic Products Corporation, Los Angeles, CA. 1989.

[10] Apex Automatic Gamma Counter, Micromedic Systems, Inc., Horsham, PA.

[11] Courtesy of the Department of Poultry Science, North Carolina State University, Raleigh, NC.

Counting the tube in a gamma counter resulted in a number inversely related to the concentration of gastrin present in the sample.

In all three RIAs data reduction occured via the logit-log representation.

HPLC Methodology. In this analysis, catecholamines were adsorbed onto alumina directly from plasma at pH 8.5. After agitation, the alumina was washed and aspirated twice with water, followed by elution with a small volum of acid. This acid served as the injectable extract. Sodium octyl sulfate, acting as a dynamic ion exchanger, was used to adjust the reverse phase separation to the desired selctivity. Adjustment of the reverse phase, combined with adjustment of the mobile phase pH, separation was optimized for norepinephrine and epinephrine.[12]

Statistical Analysis.[13] It was desirable to predict calving score using a "statistically defensible" function of the explanatory variables. Additionally, it was desirable to identify the nature of the relationship between epinephrine and gastrin concentrations. Thus regression was the statistical method of choice. Potential explanatory variables of interest were identified. These variables were epinephrine, norepinephrine, gastrin, cortisol, and T_3.

---

[12]LCEC Application Note No. 14. Bioanalytical Systems, Inc., 2701 Kent Ave., West Lafayette, IN.

[13]SAS Institute, Inc. Cary, NC. 1985.

In the statistical analysis, these terms, the associated quadratic terms, and all 2-way interactions were considered. The number of observations did not permit the evaluation of a more complex model.

Two way graphs of the variables were constructed to allow visual examination of the relationship between variables. A backward selection procedure using Mallow's C_p[14] statistic as the criterion was used to identify the most appropriate regression model. The resulting model was required to be hierarchical. That is, if a quadratic term was in the model then the linear term must have also been included. Likewise, if an interaction term was included in the model, the corresponding linear terms must have also been included.

The resulting model was then examined for influence statistics using Cook's D statistic[15]. Data that were determined to be "overly influential observations" were deleted.

Backward selection was then applied to the remaining observations. Some of the terms determined to be significant in the first backward selection dropped out because they were artifacts of the overly influential observations. This procedure yielded the final regression model.

In conducting the regression, models that were not

---

[14]Mallows, C.L. "Some Comments on C_p," Technometrics, 15, 661-675. 1973.

[15]Cook, R.D. "Detection of Influential Observations in Linear Regression," Technometrics, 19, 15-18. 1977.

considered were those that were not hierarchical or those whose error degrees of freedom were too small for the results to be considered meaningful or reliable. Additionally, models with a low R^2 or with a statistically non-significant overall F-value were not considered.

RESULTS

Significant regression equations were derived expressing the relationship between calving score and the explanatory variables of epinephrine, norepinephrine, cortisol, T_3, and gastrin for the 1990 heifers at parturition, the 1990 calves at parturition, and the 1991 calves at parturition. Additionally, a significant model was derived using the 1990 heifers and calves, relating both dam and offspring factors to calving score. Three dimensional graphs of the interactions were constructed. Graphs of the statistically significant interactions (Figures 1-7) are presented here. The remaining interaction graphs are presented in the Appendix. Analysis of Variance (ANOVA) tables are presented for each regression equation (Tables 1-4). Mean values ($\pm$ standard error of mean) for blood hormones and hemograms are presented in Tables 5 to 16.

The level of dystocia associated with each birth results in changes in blood chemistry. However, the relationship is presented here in the reverse fashion, that is as blood hormone levels causing calving score rather than calving score being the causal agent and acting upon blood parameters. This "reverse" presentation occurs as a result of developing the model from the available data.

1990 Heifers at Parturition.

Regression Equation and Significant Interactions. The regression equation (Equation 1) derived using data collected from the 1990 heifers at parturition is the following:

Equation 1:

$$\text{Score} = 5.9340 + 0.0550\text{Epi} + 0.0558\text{Nor} - 0.2566\text{Cort} - 0.0097T_3 - 0.0020\text{Gas} - 0.0002\text{Epi}^2 - 0.0009\text{Nor}^2 + 0.0000004\text{Gas}^2 + 0.0008(\text{EpiXNor}) + 0.0032(\text{EpiXCort}) + 0.00001(\text{EpiXGas})$$

Epi = Heifer Epinephrine
Nor = Heifer Norepinephrine
Cort = Heifer Cortisol
T_3 = Heifer Triiodothyronine (T_3)
Gas = Heifer Gastrin

The associated ANOVA table and R^2 value are shown in Table 1 (page 47). Superscripts of 2 indicate the quadratic effect of the superscripted variable and notation such as (EpiXNor) indicates the interaction between the two variable (in this case, epinephrine and norepinephrine).

This model was significant at the $P<0.01$ level. Significant interactions occurred between epinephrine and gastrin ($P<0.01$) and epinephrine and norepinephrine ($P<0.08$). The quadratic effect of gastrin was significant at the $P<0.01$ level. Significant main effects included epinephrine at the $P<0.06$ level, gastrin at the $P<0.06$ level, and T_3 at the $P<0.03$ level.

Epinephrine and Gastrin Interaction. The interaction between epinephrine and gastrin was significant at the $P<0.01$ level. A graphic representation of the interaction is

presented in Figure 1 (page 48). In this graph, it is possible to see that at medium levels (82-151 ng/ml) of epinephrine, increases in gastrin result in a higher calving score. However, as epinephrine increases from medium levels (82-151 ng/ml), calving score falls. If gastrin continues to decrease and epinephrine increases, calving score falls. At low levels of epinephrine, as gastrin decreases, calving score also decreases.

Epinephrine and Norepinephrine Interaction. The interaction between epinephrine and norepinephrine was significant at the $P<0.08$ level. This interaction is graphically represented in Figure 2 (page 49). In this figure a direct relationship between epinephrine and norepinephrine can be seen. As levels of epinephrine increase, levels of norepinephrine also increase. Calving score peaks at intermediate levels of norepinephrine and low levels of epinephrine. At low levels of norepinephrine, calving score is low, and calving score increases as norepinephrine concentrations increase up to a point after which calving scores fall. This pattern is consistent over all levels of epinephrine.

1990 Calves at Parturition

Regression Equation and Significant Interactions. The regression equation (Equation 2) derived from data collected from the 1990 calves at parturition is as follows:

Equation 2:

$$\text{Score} = -1.7661 + 0.2822\text{Epi} + 0.1418\text{Nor} - 0.0732\text{Cort} + 0.0004\text{Gas} + 0.0002T_3 - 0.0050\text{Epi}^2 - 0.0053(\text{EpiXCort}) - 0.0005(\text{CortX}T_3) - 0.000001(\text{Gas}x T_3)$$

Epi = Calf Epinephrine
Nor = Calf Norepinephrine
Cort = Calf Cortisol
Gas = Calf Gastrin
T_3 = Calf Triiodothyronine (T_3)

The associated ANOVA table and R^2 value are shown in Table 2 (page 50). Superscripts of 2 indicate the quadratic effect of the superscripted variable and notation such as (EpiXCort) indicates the interaction between the two variables (in this case, epinephrine and cortisol).

This model was significant at the $P<0.0001$ level. Significant interactions occurred between cortisol and T_3, norepinephrine and T_3; and norepinephrine and gastrin. The quadratic effect of epinephrine was significant at the $P<0.02$ level. Significant main effects included T_3 at the $P<0.01$ level, gastrin at the $P<0.0001$ level and epinephrine at the $P<0.01$ level.

Cortisol and T_3 Interaction. The interaction between cortisol and T_3 was significant at the $P<0.002$ level. A graphic representation of this interaction is presented in Figure 3 (page 51). In the figure, as cortisol is decreased, calving score increases with T_3 acting to decrease calving score. However, at high levels of both cortisol and T_3,

calving score is increased dramatically.

Norepinephrine and T_3 Interaction. This interaction was significant at the $P<0.09$ level. The interaction is graphically shown in Figure 4 (page 52). As norepinephrine is increased and T_3 decreased, calving score increases. However, at high levels of T_3 calving score begins to increase once again. At intermediate levels of T_3 and intermediate levels of norepinephrine, calving score begins to once again go down.

Norepinephrine and Gastrin Interaction. The norepinephrine and gastrin interaction was significant at the $P<0.003$ level. A graph indicating the interaction is presented in Figure 5 (page 53). In this graph, as norepinephrine is increased and gastrin is decreased calving score increases. At intermediate levels of gastrin and norepinephrine, calving score peaks and proceeds to decline.

1990 Heifers and Calves At Birth (combined regression)

Regression Equation and Significant Interactions. The regression equation (Equation 3) derived utilizing both data collected from the 1990 heifers at parturition and the 1990 calves at parturition is as follows:

Equation 3:

$$\text{Score} = 1.6846 + 0.0011\text{Cepi} + 0.0062\text{Cfepi} + 0.0001\text{Cfgas} - 0.0000001\text{Cfgas}^2$$

Cepi = Heifer Epinephrine
Cfepi = Calf Epinephrine
Cfgas = Calf Gastrin
Cfgas^2 = Quadratic effect of Calf Gastrin

The associated ANOVA table and R^2 value are shown in Table 3 (page 54).

This model was significant at the $P<0.004$ level. The main effect of calf gastrin was significant at the $P<0.0008$ level. The heifer epinephrine was also significant at the $P<0.07$ level. There were no significant interactions present in this model.

1991 Calves at Parturition

Regression Equation and Significant Interactions. The regression equation (Equation 4) derived utilizing the data collected from the 1991 calves at parturition is as follows:

Equation 4:

$$\begin{aligned} \text{Score} = & -3.5297 - 0.0103\text{Epi} + 0.0082\text{Nor} + \\ & 0.3364\text{Cort} + 0.0342\text{Gas} + 0.0139T_3 - \\ & 0.00002\text{Gas}^2 - 0.00001T_3^2 + \\ & 0.0002(\text{EpiXGas}) - 0.0022(\text{CortXGas}) - \\ & 0.0008(\text{CortX}T_3) \end{aligned}$$

Epi = Calf Epinephrine
Nor = Calf Norepinephrine
Cort = Calf Cortisol
Gas = Calf Gastrin
T_3 = Calf Triiodothyronine (T_3)

The associated ANOVA table and R^2 values are shown in table 4 (page 55). Superscripts of 2 indicate the quadratic effect of the superscripted variable and notation such as (EpiXGas) indicates the interaction between the two variables (in this case, epinephrine and gastrin).

This model was significant at the $P<0.01$ level. Significant interactions included cortisol and T_3 and cortisol

and gastrin. The only significant main effect was T_3 at a level of $P<0.005$.

Cortisol and T_3 Interaction. The cortisol and T_3 interaction was significant at the level of $P<0.07$. This interaction is graphically shown in Figure 6 (page 56). At approximately 380 ng/dl of T_3 and lower, as cortisol concentrations increase, calving scores decrease. However, from concentrations of cortisol of 15 ug/dl and lower, as T_3 levels are increased to approximately 700 ng/ml, calving score increases. At greater T_3 concentrations, calving scores decrease.

Cortisol and Gastrin Interaction. The interaction between cortisol and gastrin was significant at the $P<0.006$ level. It is graphically represented in Figure 7 (page 57). In this figure it can be seen that as cortisol increases calving score decreases as long as gastrin remains below 300 pg/ml. If gastrin levels are above 300 pg/ml, as cortisol concentrations decrease, calving score increases up to a maximum, after which it decreases again. At intermediate to lower levels of cortisol, increasing gastrin concentrations result in increased calving scores.

Comparison of Regression Equations

In comparing the regression models, Equation 2 is the best fit for prediction of calving score based on the variables of interest. The R^2 values for Equation 1 and

Equation 2 are very similar.

Although the three models derived from the 1990 data set (Equations 1, 2, and 3) possess the strongest R^2, the best predictive equation may be Equation 4. Gastrin values found in the 1990 serum were approximately 10-fold greater than those previously reported in cattle (Rainforth, unpublished data). The gastrin in 1991 more closely resemble those reported by Rainforth.

Hemogram Means and Blood Hormone Means

Hemogram means and blood hormone means are presented in Tables 5 to 16. The means and their associated standard errors are reported on the heifers at parturition, calves at parturition and 24 hours of age for both 1990 and 1991. Due to the effects of the interaction described previously, significant differences are not reported on the blood hormone mean values.

Table 1: Analysis of Variance
for 1990 Heifers at Parturition

Source	Degrees Of Freedom	Type1 Sums of Squares	F-Value	Pr >F
Model	11	3.9470	3.30	0.0134
Epinephrine	1	0.0458	4.22	0.0556
Norepinephrine	1	0.0025	0.02	0.8819
Cortisol	1	0.0005	0.00	0.9452
T_3	1	0.6079	5.60	0.0301
Gastrin	1	0.4341	4.00	0.0618
Epi X Epi	1	0.0065	0.06	0.8102
Nor X Nor	1	0.2835	2.61	0.1246
Gas X Gas	1	0.8187	7.54	0.0138
Epi X Nor	1	0.3880	3.57	0.0759
Epi X Cort	1	0.0243	0.22	0.6422
Epi X Gas	1	0.9226	8.50	0.0097
Error	17	1.8431		
Total	28	5.7931		

$R^2 = 0.6813$

Figure 1: Gastrin X Epinephrine Interaction
in 1990 Heifers at Parturition

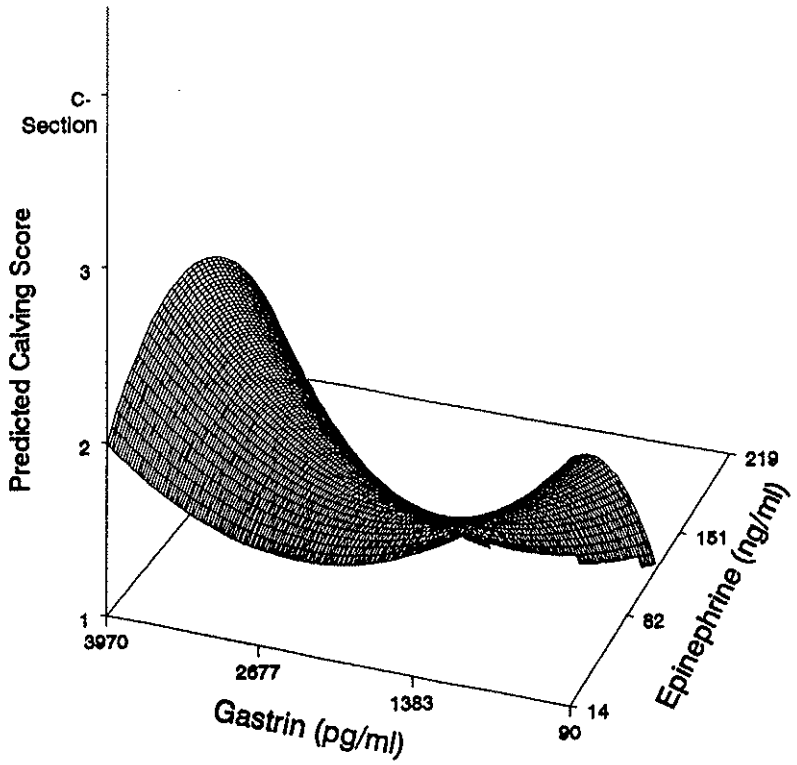


Figure 2: Epinephrine X Norepinephrine Interaction
For 1990 Heifers at Parturition

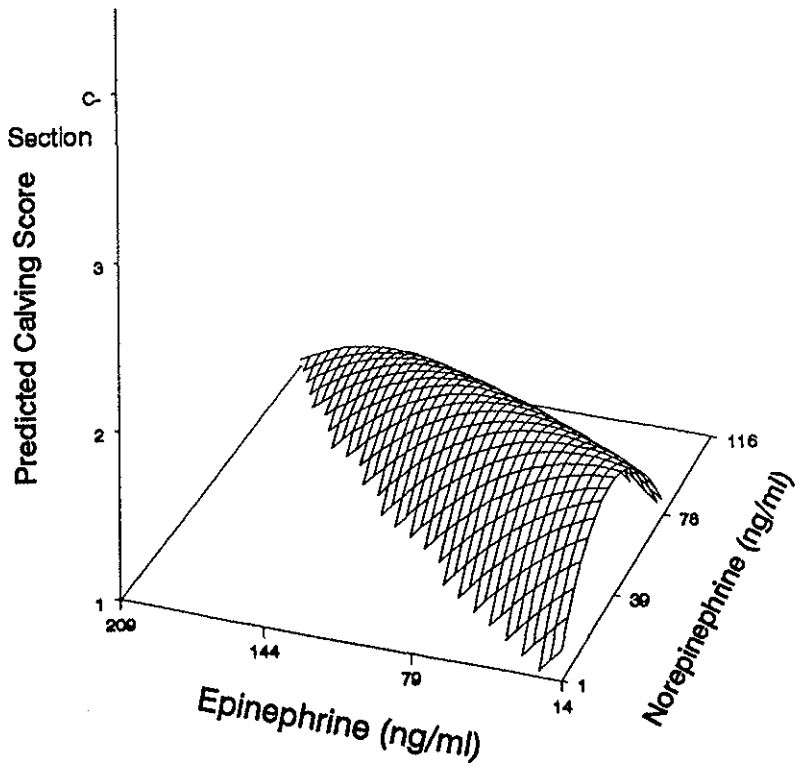


Table 2: Analysis of Variance
for 1990 Calves At Parturition

Source	Degrees Of Freedom	Type1 Sums of Squares	F-Value	Pr >F
Model	11	10.7608	6.43	0.0001
Epinephrine	1	1.1547	7.59	0.0095
Norepinephrine	1	0.1542	1.01	0.3212
Cortisol	1	0.0028	0.02	0.8935
Gastrin	1	3.0554	20.10	0.0001
T_3	1	1.0711	7.05	0.0121
Epi X Epi	1	0.8695	5.72	0.0226
Epi X Cort	1	0.3428	2.26	0.1427
Nor X Gas	1	1.5312	10.07	0.0033
Nor X T_3	1	0.4694	3.09	0.0882
Cort X T_3	1	1.7040	11.21	0.0020
Gas X T_3	1	0.4057	2.67	0.1118
Error	33	5.0170		
Total	44	15.7778		

$R^2 = 0.6820$

Figure 3: Cortisol X T3 Interaction
for 1990 Calves at Parturition

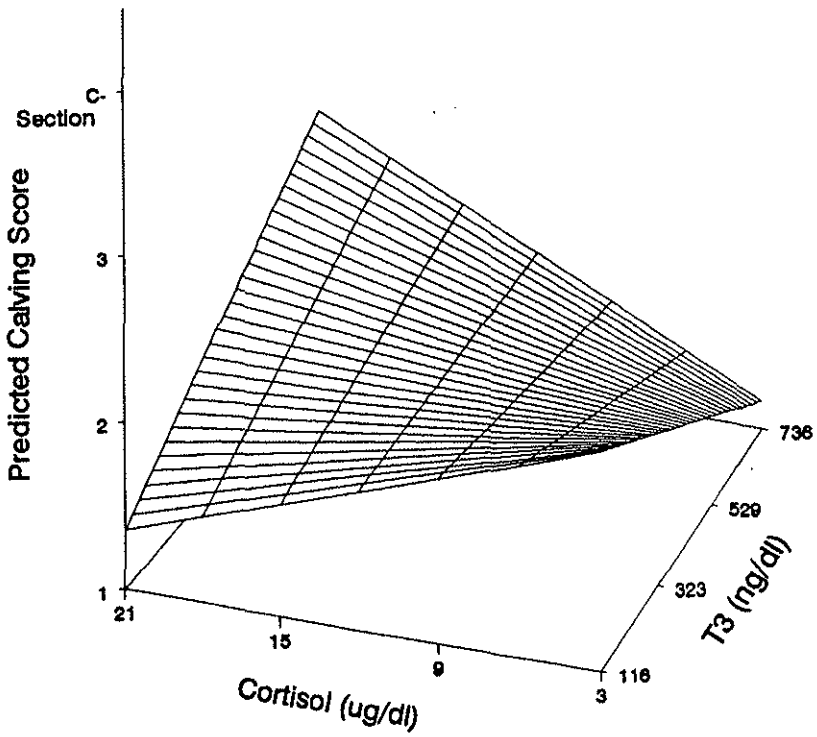


Figure 4: T3 X Norepinephrine Interaction
for 1990 Calves at Parturition

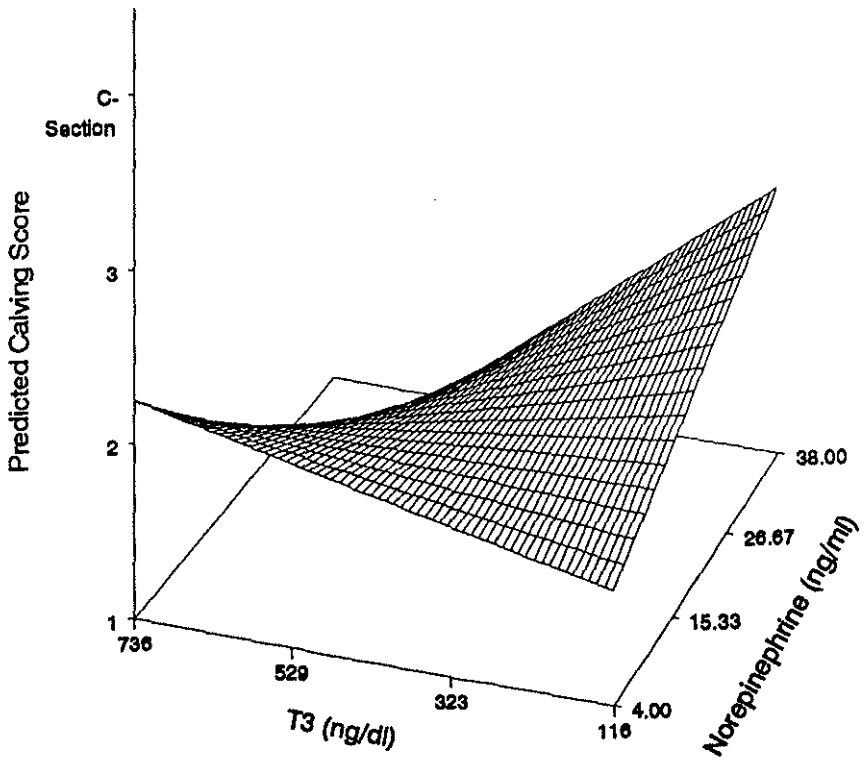


Figure 5: Gastrin X Norepinephrine
Interaction for 1990 Calves at Parturition

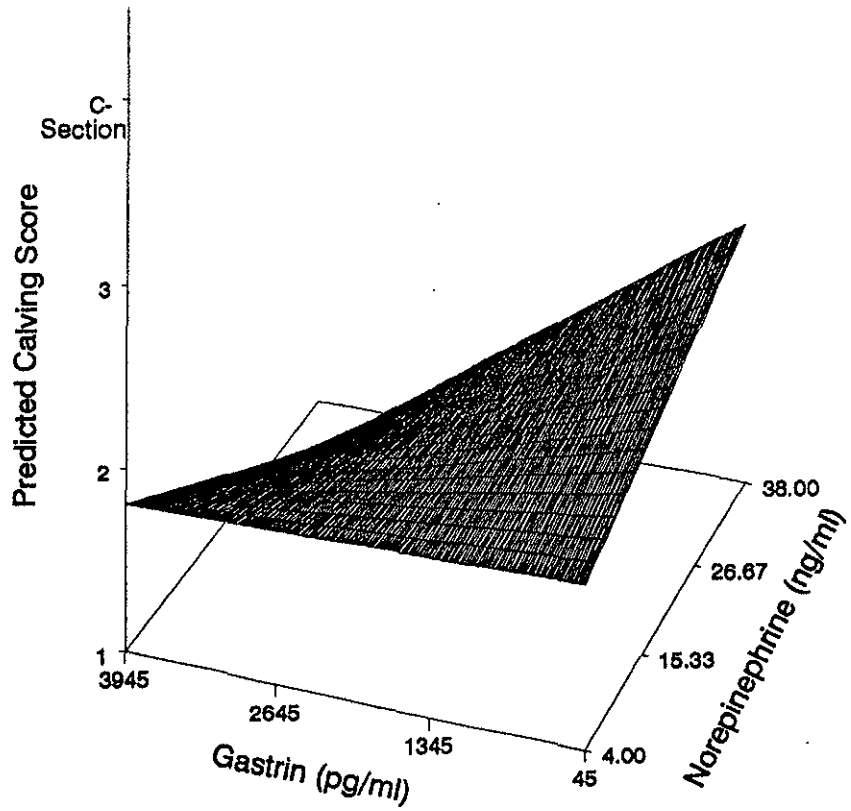


Table 3: Analysis of Variance for 1990 Heifers and Calves at Parturition

Source	Degrees Of Freedom	Type1 Sums of Squares	F-Value	Pr >F
Model	4	2.7462	5.82	0.0035
Heifer Epinephrine	1	0.4556	3.86	0.0650
Calf Epinephrine	1	0.1047	0.89	0.3586
Calf Gastrin	1	1.8996	16.10	0.0008
Calf Gastrin X Calf Gastrin	1	0.2864	2.43	0.1366
Error	18	2.1233		
Total	22	4.8696		

$R^2 = 0.5640$

Table 4: Analysis of Variance
for 1991 Calves at Parturition

Source	Degrees Of Freedom	Typel Sums of Squares	F-Value	Pr >F
Model	10	10.3961	3.16	0.0117
Epinephrine	1	0.8578	2.61	0.1207
Norepinephrine	1	0.6660	2.02	0.1689
Cortisol	1	0.8465	2.57	0.1230
Gastrin	1	0.2900	0.87	0.3605
T_3	1	3.1512	9.58	0.0053
Epi X Epi	1	0.0487	0.15	0.7042
T_3 X T_3	1	0.0159	0.05	0.8282
Epi X Gas	1	0.2282	0.69	0.4140
Cort X Gas	1	3.1113	9.45	0.0055
Cort X T_3	1	1.1836	3.60	0.0711
Error	22	7.2402		
Total	32	17.6364		

$R^2 = 0.5895$

Figure 6: Cortisol X T3 Interaction
for 1991 Calves at Parturition

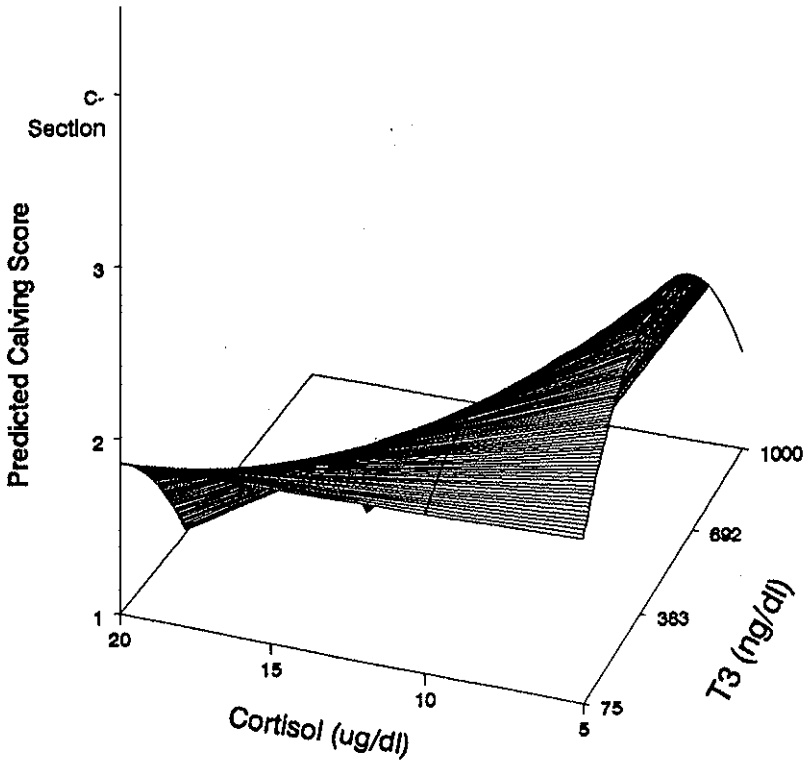


Figure 7: Cortisol X Gastrin Interaction
for 1991 Calves at Parturition

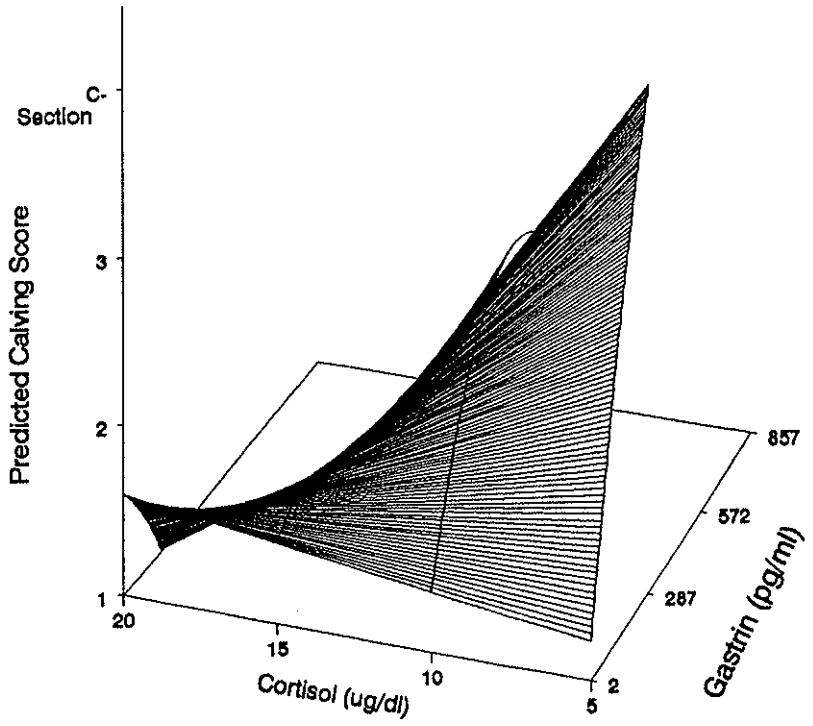


Table 5: Plasma hormone and osmolarity mean values on 1990 heifers at calving

Calving Score[1]	1	2	3	C-Section	Mal-presented
Epinephrine (ng/ml)	30.18 (18.64)[3]	63.54 (11.50)	ND[2]	ND[2]	86.99 (24.16)
Norepinephrine (ng/ml)	43.24 (10.23)	49.89 (5.83)	ND[2]	21.12 ------[4]	65.85 (15.67)
T_3 (ng/dl)	191.70 (11.11)	199.64 (6.84)	285.03 -------	165.55 ------	206.49 (15.22)
Cortisol (ug/dl)	3.84 (0.43)	4.33 (0.27)	4.27 -------	1.78 ------	5.15 (0.60)
Gastrin (pg/ml)	2014.14 (316.84)	1385.45 (194.90)	400.07 -------	415.55 ------	1579.18 (462.37)
Osmolarity (mOsm/kg)	290.57 (3.44)	284.01 (2.12)	298.50 -------	286.00 ------	290.29 (4.87)
Number of Heifers	14	36	1	1	7

1. Calving Scores Based on Grouping of MARC Scores as Follows:

Score	MARC Score
1	Unassisted or hand pull
2	Mechanical pull
3	Severe mechanical pull

2. ND indicates hormone not detectable within ranges of instrumentation.

3. Standard error of mean indicated by number in parentheses.

4. Standard Error of mean not reported when $N \leq 2$.

Table 6: Plasma hormone and osmolarity mean values on 1990 calves at birth

Calving Score[1]	1	2	3	C-Section	Mal-Presented
Epinephrine (ng/ml)	18.39 (1.92)[2]	21.83 (1.26)	23.59 -----[3]	27.45 -----	22.57 (3.34)
Norepinephrine (ng/ml)	14.78 (2.03)	14.65 (1.32)	24.53 -----	25.33 -----	19.04 (3.21)
T_3 (ng/dl)	383.25 (28.01)	299.72 (17.72)	389.86 -----	220.93 -----	271.56 (39.46)
Cortisol (ug/dl)	9.19 (1.02)	9.13 (0.64)	8.46 -----	7.73 -----	10.68 (1.61)
Gastrin (pg/ml)	2102.51 (293.97)	1114.04 (185.93)	56.14 -----	468.41 -----	1621.61 (477.45)
Osmolarity (mOsm/kg)	271.71 (4.14)	277.40 (2.62)	320.00 -----	277.50 -----	291.75 (6.32)
Number of Calves	14	36	1	1	7

1. See Endnote 1, Table 5

2. Standard error of mean indicated by number in parentheses.

3. See Endnote 3, Table 5.

Table 7: Plasma hormone and osmolarity mean values on 1990 calves at 24 hours of age

Calving Score[1]	1	2	3	C-Section	Mal-Presented
Epinephrine (ng/ml)	19.99 (3.00)[2]	21.03 (2.03)	------	21.28 -----[3]	25.81 (6.29)
Norepinephrine (ng/ml)	11.43 (2.26)	13.93 (1.57)	------	5.47 ------	14.35 (1.76)
T_3 (ng/dl)	468.51 (45.27)	563.83 (29.95)	------	406.26 ------	449.72 (50.55)
Cortisol (ug/dl)	3.93 (0.49)	4.81 (0.32)	------	4.85 ------	3.67 (1.45)
Gastrin (pg/ml)	3433.79 (467.17)	1146.91 (309.00)	------	470.32 ------	2416.35 (1377.19)
Osmolarity (mOsm/kg)	270.32 (2.84)	268.75 (1.88)	------	271.50 ------	268.75 (5.30)
Number of Calves	14	30	0	1	4

1. See Endnote 1, Table 5

2. Standard error of mean indicated by number in parentheses.

3. See Endnote 3, Table 5.

Table 8: Mean differential leukocyte counts for 1990 heifers at calving

Calving Score[1]	1	2	3	C-Section	Mal-Presented
Eosinophils (%)	0 (0.26)[2]	0.41 (0.14)	0 -----[3]	0 ------	0 (0.36)
Basophils (%)	0 (0)	0 (0)	0 ------	0 ------	0 (0)
Monocytes (%)	0.36 (0.92)	1.46 (0.50)	0 ------	0 ------	0.33 (1.24)
Lymphocytes(%)	38.09 (3.87)	39.57 (2.11)	44.00 ------	19.00 ------	40.00 (5.24)
Neutrophils (%)					
Mature	60.36 (3.77)	57.46 (2.06)	53.00 ------	76.00 ------	58.50 (5.11)
Band[4]	1.18 (0.35)	1.11 (0.19)	3.00 ------	5.00 ------	1.17 (0.47)
N:L Ratio	1.80 (0.99)	2.28 (0.54)	1.20 ------	4.20 ------	1.65 (1.33)
Number of Heifers	14	36	1	1	7

1. See Endnote 1, Table 5

2. Standard error of mean indicated by number in parentheses.

3. See Endnote 3, Table 5.

4. P<0.0027 Score 1 vs. Caesarean
   P<0.0018 Score 2 vs. Caesarean
   P<0.0034 Caesarean vs. Malpresentation

Table 9: Mean differential leukocyte counts for 1990 calves at birth

Calving Score[1]	1	2	3	C-Section	Mal-Presented
Eosinophil (%)	0.07 (0.05)[2]	0.03 (0.03)	0 ----[3]	0 -----	0 (0.08)
Basophils (%)	0 (0)	0 (0)	0 -----	0 -----	0 (0)
Monocytes (%)	0.79 (0.31)	0.43 (0.19)	1.00 -----	0 -----	0.33 (0.47)
Lymphocytes (%)[4]	36.79 (3.83)	39.54 (2.42)	26.00 -----	31.00 -----	50.00 (5.86)
Neutrophils (%)					
Segmented[5]	60.71 (3.96)	58.31 (2.50)	68.00 -----	69.00 -----	46.67 (6.04)
Banded[6]	1.64 (0.35)	1.40 (0.22)	5.00 -----	0 -----	1.33 (0.54)
N:L Ratio[7]	1.77 (0.30)	1.89 (0.19)	2.80 -----	2.20 -----	1.04 (0.43)
Number of Calves	14	36	1	1	7

1. See Endnote 1, Table 5

2. Standard error of mean indicated by number in parentheses.

3. See Endnote 3, Table 5.

4. P<0.0245 Score 1 vs. Malpresentation
   P<0.0412 Score 2 vs. Malpresentation
   P<0.0999 Score 3 vs. Malpresentation

5. P<0.0307 Malpresentation vs. Score 1
   P<0.0553 Malpresentation vs. Score 2

6. P<0.0139 Score 1 vs. Score 3
   P<0.0071 Score 2 vs. Score 3
   P<0.0089 Score 3 vs. Caesearan

7. P<0.0857 Score 2 vs. Malpresentation

Table 10: Mean differential leukocyte counts for 1990 calves at 24 hours of age

Calving Score[1]	1	2	3	C-Section	Mal-Presented
Eosinophil (%)	0.14 (0.10)[2]	0.13 (0.07)	------	0 ----[3]	0 (0.19)
Basophils (%)	0 (0)	0 (0)	------	0 -----	0 (0)
Monocytes (%)[4]	1.43 (0.40)	0.47 (0.27)	------	0 -----	0.50 (0.75)
Lymphocytes (%)	38.71 (3.98)	33.97 (2.63)	------	51.00 -----	35.00 (7.44)
Neutrophils (%)					
Mature	58.64 (3.73)	65.34 (2.47)	------	49.00 -----	64.25 (6.98)
Band	1.07 (0.39)	1.28 (0.26)	------	0 -----	0.25 (0.72)
N:L Ratio[5]	1.82 (0.39)	2.52 (0.26)	------	0.90 -----	1.85 (0.74)
Number of Calves	14	30	0	1	4

1. See Endnote 1, Table 5

2. Standard error of mean indicated by number in parentheses.

3. See Endnote 3, Table 5.

4. $P<0.059$ Score 1 vs. Score 2

5. $P<0.08$ Score 1 vs. Score 2

Table 11: Plasma hormone and osmolarity mean values on 1991 heifers at calving

Calving Score[1]	1	2	3	C-Section	Mal-Presented
Epinephrine (ng/ml)	43.34 (12.29)[3]	72.38 (18.24)	ND[2]	88.45 -----[4]	ND[2]
Norepinephrine (ng/ml)	102.64 (15.35)	97.77 (14.79)	67.64 (45.20)	171.28 ------	23.47 (38.82)
T_3 (ng/dl)	148.90 (5.45)	135.69 (5.45)	113.25 (16.65)	153.11 ------	103.41 (13.51)
Cortisol (ug/dl)	4.38 (0.31)	6.77 (0.31)	7.09 (0.94)	4.20 ------	6.05 (0.78)
Gastrin (pg/ml)	94.23 (8.17)	121.62 (8.03)	167.29 (24.98)	127.14 ------	171.65 (20.90)
Osmolarity (mOsm/kg)	288.63 (3.83)	305.91 (3.83)	285.33 (11.69)	308.50 ------	317.88 (10.13)
Number of Heifers	28	30	3	1	4

1. See Endnote 1, Table 5.

2. See Endnote 2, Table 5.

3. Standard error of mean indicated by number in parentheses.

4. See Endnote 3, Table 5.

Table 12: Plasma hormone and osmolarity mean values on 1991 calves at birth

Calving Score[1]	1	2	3	C-Section	Mal-Presented
Epinephrine (ng/ml)	17.92 (2.60)[2]	21.37 (2.68)	36.26 (8.03)	13.45 -----[3]	33.51 (11.43)
Norepinephrine (ng/ml)	49.76 (7.62)	61.38 (7.47)	70.86 (21.99)	98.41 ------	55.89 (17.93)
T_3 (ng/dl)	459.81 (33.07)	241.75 (33.07)	252.12 (101.03)	245.47 ------	238.77 (85.49)
Cortisol (ug/dl)	12.44 (0.54)	13.24 (0.54)	10.63 (1.66)	9.07 ------	11.59 (1.43)
Gastrin (pg/ml)	89.85 (36.24)	152.62 (35.59)	283.19 (108.73)	129.38 ------	316.40 (97.57)
Osmolarity (mOsm/kg)	271.28 (4.59)	282.04 (4.50)	275.33 (13.76)	267.50 ------	276.50 (11.92)
Number of Calves	25	29	3	1	4

1. See Endnote 1, Table 5.

2. Standard error of mean indicated by number in parentheses.

3. See Endnote 3, Table 5.

Table 13: Plasma hormone and osmolarity mean values on 1991 calves at 24 hours of age

Calving Score[1]	1	2	3	C-Section	Mal-Presented
Epinephrine (ng/ml)	29.27 (8.44)[3]	17.22 (10.90)	11.87 (26.70)	ND[2]	11.87 (27.69)
Norepinephrine (ng/ml)	36.72 (5.17)	38.72 (5.72)	50.52 (14.01)	ND[2]	37.15 (14.47)
T_3 (ng/dl)	533.11 (28.26)	447.45 (28.26)	305.99 (86.35)	392.63 ------[4]	326.92 (74.99)
Cortisol (ug/dl)	5.10 (0.41)	5.41 (0.41)	6.36 (1.26)	3.32 ------	6.23 (1.09)
Gastrin (pg/ml)	162.51 (28.01)	116.02 (27.50)	351.97 (84.02)	213.85 ------	388.03 (86.29)
Osmolarity (mOsm/kg)	276.54 (3.80)	272.24 (3.80)	281.50 (11.41)	278.50 ------	281.63 (9.88)
Number of Calves	24	28	3	1	4

1. See Endnote 1, Table 5.

2. See Endnote 2, Table 5.

3. Standard error of mean indicated by number in parentheses.

4. See Endnote 4, Table 5.

Table 14: Mean differential leukocyte counts for 1991 heifers at calving

Calving Score[1]	1	2	3	C-Section	Mal-Presented
Eosinophil (%)[2]	0.50 (0.20)[3]	0.45 (0.19)	0 (0.58)	3.00 -----[4]	0 (0.50)
Basophils (%)	0.04 (0.07)	0.14 (0.06)	0 (0.19)	0 -----	0 (0.17)
Monocytes (%)[5]	4.38 (0.80)	4.03 (0.76)	1.33 (2.37)	0 -----	0.50 (2.05)
Lymphocytes (%)[6]	39.50 (3.22)	33.21 (3.05)	33.33 (9.48)	61.00 -----	32.00 (8.21)
Neutrophils (%)					
Mature	54.31 (3.26)	59.24 (3.08)	63.00 (9.59)	36.00 -----	65.75 (8.30)
Band	1.65 (0.66)	2.93 (0.63)	3.33 (1.95)	0 -----	2.50 (1.69)
N:L Ratio	2.13 (0.30)	2.35 (0.28)	1.97 (0.87)	0.50 -----	2.10 (0.76)
Number of Heifers	28	30	3	1	4

1. See Endnote 1, Table 5.

2. P<0.02 Score 1 vs. Caesarean
   P<0.01 Score 2 vs. Caesarean
   P<0.01 Score 3 vs. Caesarean
   P<0.008 Caesarean vs. Malpresentation

3. Standard error of mean indicated by number in parentheses.

4. See Endnote 4, Table 5.

5. P<0.08 Score 1 vs Malpresentation

6. P<0.097 Score 2 vs. Caesarean
   P<0.1163 Malpresentation vs. Caesarean

Table 15: Mean differential leukocyte counts for 1991 calves at birth

Calving Score[1]	1	2	3	C-Section	Mal-Presented
Eosinophil (%)	0.58 (0.17)[2]	0.39 (0.17)	0 (0.49)	0 -----[3]	0 (0.42)
Basophils (%)	0.23 (0.15)	0.32 (0.14)	0 (0.44)	0 -----	0.25 (0.38)
Monocytes (%)[4]	4.08 (0.84)	3.71 (0.81)	0.33 (2.48)	1.00 -----	0.25 (2.15)
Lymphocytes (%)	39.19 (4.37)	39.35 (4.21)	55.00 (12.86)	31.00 -----	35.00 (11.14)
Neutrophils (%)					
Mature	54.19 (4.34)	54.64 (4.19)	41.00 (12.80)	63.00 -----	61.75 (11.08)
Band	1.77 (0.66)	2.96 (0.63)	3.67 (1.94)	5.00 -----	4.25 (1.68)
N:L Ratio	2.31 (0.39)	2.31 (0.37)	1.40 (1.14)	2.10 -----	1.95 (0.99)
Number of Calves	25	29	3	1	4

1. See Endnote 1, Table 5.

2. Standard error of mean indicated by number in parentheses.

3. See Endnote 4, Table 5.

4. $P<0.08$ Score 2 vs. Malpresentation

Table 16: Mean differential leukocyte counts for 1991 calves at 24 hours of age

Calving Score[1]	1	2	3	C-Section	Mal-Presented
Eosinophil (%)	0.40 (0.26)[2]	0.77 (0.25)	0.33 (0.75)	1.00 -----[3]	0.50 (0.65)
Basophils (%)	0 (0.04)	0.08 (0.04)	0 (0.11)	0 -----	0 (0.09)
Monocytes (%)[4]	2.80 (0.47)	2.46 (0.46)	6.67 (1.36)	0 -----	4.00 (1.18)
Lymphocytes (%)	36.72 (3.73)	33.81 (3.65)	48.67 (10.76)	71.00 -----	38.25 (9.32)
Neutrophils (%)					
Mature	56.92 (3.66)	59.15 (3.58)	35.33 (10.55)	28.00 -----	49.00 (9.14)
Band	3.12 (1.04)	3.73 (1.02)	9.00 (3.01)	0 -----	8.25 (2.60)
N:L Ratio	3.21 (0.89)	2.95 (0.87)	0.96 (2.57)	0.30 -----	1.73 (2.23)
Number of Calves	24	28	3	1	4

1. See Endnote 1, Table 5

2. Standard error of mean indicated by number in parentheses.

3. See Endnote 4, Table 5.

4. $P<0.08$ Score 2 vs. Malpresentation

Discussion

Stress is well accepted as a fact of day to day life. Cannon and Selye both described the response of the body to stress (12, 82). Since these early investigations much work has been done attempting to quantify stress and develop objective methods for stress assessment (35).

In response to stress, there is an increase in plasma glucocorticoid hormone concentrations, release of epinephrine from the adrenal medulla, norepinephrine from the sympathetic nerves and corticosteroids from the adrenal cortex (3, 8). Each of these hormones then stimulate a cascade of events contributing to the overall stress response. This response varies depending upon the source and the intensity of the stressor (4, 17, 24, 27, 31, 34, 38, 60, 61, 62, 63). The typical physiological responses to stress include increases in heart rate, blood pressure, and metabolism (2, 35, 92).

In a production situation, the level of calving difficulty is often assigned a calving score. There is some question as to whether or not the score assigned by the herdsman actually reflects the stress level of the calf. It is important to realize the impact of the stress of delivery. This may allow earlier intervention on calves that may be suffering from perinatal maladjustment due to calving stress.

Catecholamines

In most adult mammals, the main catecholamine produced is epinephrine (3). However, it is well established that the primary fetal catecholamine is norepinephrine (30, 51, 54, 64). Epinephrine is synthesized from norepinephrine, a reaction promoted by the enzyme PNMT (41).

A wide range of catecholamine levels occurred across both 1990 and 1991 data groups. However, overall, the 1991 norepinephrine values (Tables 11, 12, 13, pages 64, 65, 66) tended to be higher. Due to analytical and instrumental difficulties, analysis of the 1990 catecholamine samples was delayed. Metabolic destruction of norepinephrine may explain the lower norepinephrine values detected in 1990 (Tables 5, 6, 7, pages 58, 59, 60). Due to the delay in analysis, a direct comparison is not made between the two years of data.

As expected, in the 1991 calves, norepinephrine levels were higher than the epinephrine levels (Table 12, page 65). However, the norepinephrine levels were also higher than the epinephrine levels in the 1991 heifers (Table 11, page 64). Additionally, as calving score increased, the level of norepinephrine in these heifers decreased. This may have occurred as a result of increased conversion of norepinephrine to epinephrine by the enzyme PNMT, as the calving score, and potentially the stress, increased. Epinephrine levels at a

calving score of 3 and a malpresentation were beyond the ranges of the instrumentation, thus were not quantifiable.

In examining the epinephrine levels of the calves at birth in both 1990 and 1991 (Tables 6 and 12, pages 59 and 65), it can be noticed that there is a slight increase in epinephrine levels as calving score increases from 1 to 3. During a prolonged and/or dystocial delivery, the level of hypoxia experienced by the calf may increase. As a result of anaerobic conditions, a higher percentage of the catecholamine concentration is made up by epinephrine (81). A similar pattern is exhibited in the heifer data (Table 5 and 11, pages 58 and 64) however, the epinephrine concentrations were not quantified at a calving score of 3.

Another factor that may have resulted in the increases in epinephrine levels as calving scores increased is cortisol. Cortisol has been shown to promote epinephrine release but not norepinephrine release (16, 37). For the heifers in this study, the general trend appears to be that as cortisol levels are increasing, so are epinephrine levels. As cortisol concentrations increased from 3.84 to 4.33 ug/dl in 1990 and 4.38 to 6.77 ug/dl in 1991 over calving scores 1 and 2, epinephrine conentrations increased from 30.18 to 63.54 ng/ml and 43.34 to 72.38 ng/ml in 1990 and 1991 respectively. Although not as evident from the means, a similar response is seen in the 1990 calves (Appendix Figure 1, page 100).

Cortisol

Cortisol levels found in this study are comparable to values reported by other authors (21, 29, 95). Maternal cortisol means ranged from 1.78 ug/dl to 5.15 ug/dl in 1990 (Table 5, page 58) and 4.2 ug/dl to 7.09 ug/dl in 1991 (Table 11, page 64). Calf cortisol levels ranged from 7.73 ug/dl to 10.68 ug/dl in 1990 (Table 6, page 59) and 9.07 ug/dl to 13.24 ug/dl in 1991 (Table 12, page 65). Higher plasma cortisol concentrations were seen in the calves at all calving scores. As there may be some placental transfer of cortisol from maternal to fetal circulation, the higher levels of cortisol seen in the calves may be a reflection of both maternal cortisol levels and fetal cortisol levels due to the occurrence of placental transfer. However, in order for a transfer of cortisol to occur from maternal to fetal circulation, an active transport system must be present in order to force cortisol across a concentration gradient.

With the exception of the 1991 heifers at parturition, there is no distinct pattern associated with cortisol levels. However, in heifers and calves in both 1990 and 1991, mean cortisol levels were lower immediately following the Caesarean sections. This may indicate that if a Caesarean section is performed in a timely fashion, the stress on both the dam and the calf may be reduced.

While there was little change, or a slight increase, in maternal cortisol levels when calving scores increased, calf cortisol levels fell slightly. Increases in cortisol levels are generally associated with chronic stress. From a maternal point of view, parturition could be considered an acute stress, thus resulting in little change in plasma cortisol levels. The fetal hypothalamic-pituitary axis controls parturition and fetal cortisol levels begin to increase up to 15 days prior to parturition (49, 100). Therefore, the preparation for parturition and parturition itself may be considered more of a chronic stress from the calf's point of view.

The apparent drop in calf cortisol level at a score of 3 and during a Caesarean section may not be representative due to the small number of animals sampled at these levels. However, in this study, heifers were palpated in order to determine whether or not the calf was deliverable vaginally. If a caesarean section was deemed necessary, no attempt was made to pull the calf. Thus, the calf was not stressed by a labored pull. The lowered cortisol concentrations in both the heifers and the calves in 1990 and 1991, may indicate that a Caesarean section is actually less stressful upon the animals than a difficult pull.

T_3 (Triiodothyronine)

T_3 is a phenolic amine synthesized by follicles of the thyroid gland. The primary effect of T_3 is to help control thermogenesis (70). Immediately after birth, a surge in T_3 occurs which is thought to aid the calf in adapting to the environmental temperature change that occurs when the fetus is expelled from the uterus.

Heifer mean T_3 values ranged from 165.55 ng/dl to 285.03 ng/dl in 1990 (Table 5, page 58) and 103.41 to 153.11 in 1991 (Table 11, page 64). Ambient temperatures were slightly lower during the 1990 calving season (See Appendix Weather Tables, pages 104 to 107). This may account for the difference seen between the two years of data.

Calf mean T_3 values at parturition ranged from 220.93 ng/dl to 389.86 ng/dl in 1990 (Table 6, page 59) and 238.77 ng/dl to 459.81 ng/dl in 1991 (Table 12, page 65). Calf T_3 ranges are higher than those of the heifers, as might well be expected. At parturition, calves can undergo an environmental temperature change as great as 75-100° F, so it would be appropriate for the T_3 values at parturition to be much greater than those of the heifer.

In examining the means, no distinct trend is seen between calving score and T_3 levels. However, the T_3 values for calves with a calving score of 1 was typically higher than the other calving scores. Calves with a score of 1 represent those

animals delivered unassisted or with little difficulty. Some of these animals were actually delivered in the pasture where the ambient temperature was lower than in the calving barn, where the calves with higher calving scores were delivered. As the calving barn is a relatively constant temperature, the similarity in T_3 levels between scores 2, 3, Caesarean section, and malpresentations is understandable.

Gastrin

Gastrin is a polypeptide hormone whose primary effect is to increase gastric acid secretion, promote gastrointestinal growth and increase motility of the antral portion of the stomach (41). Epinephrine is known to increase gastrin secretion (19, 47, 90). Because of this connection, epinephrine is the catecholamine associated with peptic ulcerations and stress (19, 90).

The gastrin concentrations for both 1990 heifers and calves are approximately 10-fold greater than those reported by Rainforth (75), and also those found in the 1991 heifers and calves (Tables 5, 6, 7, 11, 12, 13, pages 58 to 60 and 64 to 66). Due to laboratory and analytical difficulties, an extensive period of time passed before these samples were analyzed. Therefore, these values are considered suspect.

In 1991, the calf serum gastrin concentrations ranged from 89.85 pg/ml to 316.40 pg/ml (Table 12, page 65). The

heifer serum gastrin ranged from 94.23 pg/ml to 171.65 pg/ml (Table 11, page 64). These determined values are slightly higher than those reported by other authors (108). Overall, the calf gastrin levels tend to be higher than the heifer gastrin levels. Shulkes, et al. (84), report that in fetal sheep, gastrin levels are higher than maternal gastrin. As these samples were taken at birth, the fetal levels of gastrin may be impacting the neonatal levels.

In these gastrin levels, a slight trend is apparent. As the level of calving difficulty increased from 1 to 3, so did gastrin concentrations. This may have been a result of the increasing epinephrine concentrations that occurred over these calving scores.

Hematology

As previously indicated, the hemogram of the newborn calf, as compared to the adult animal, exhibits leukocytosis, neutrophilia, eosinopenia, and relative lymphocytopenia (29). Tennant (97) reported a mean neutrophil:lymphocyte ratio of 2.8 in calves at birth, whereas Jain (52) reported a value of 1.1 on the day of birth. An approximate neutrophil:lymphocyte ratio of 0.48 is reported in adult cattle (52) with a typical differential leukocyte count at parturition as follows:

Band Neutrophils	0-2%
Mature Neutrophils	13-68%
Lymphocytes	25-67%
Monocytes	2-12%
Eosinophils	2-9%
Basophils	0%

In both 1990 and 1991, eosinopenia was apparent in the heifers (Tables 8 and 14, pages 61 and 67) and the calves (Table 9 and 15, pages 62 and 68) at all levels of calving score. This response is a typical glucocorticoid-mediated effect (29). Additionally, lymphocyte levels appeared to be slightly depressed as compared to normal reported values for adult cattle and calves on the day of birth (52). Neutrophilia was also observed in all groups. These are both glucocorticoid-mediated effects and are associated with parturition. However, these observations are all relative to the differential counts and may not have been apparent in an absolute white cell count.

No distinctive pattern was observed between the neutrophil:lymphocyte ratio and calving score. However, heifer neutrophil:lymphocyte ratios were higher than those previously reported. In acute stress, such as parturition, the neutrophil:lymphocyte ratio increases and then decreases when the stress subsides. It may be that the reported values are "basal" and the increases seen here result from the stress of gestation and parturition.

In 1990, the neutrophil:lymphocyte ratio in the calves at birth appears to increase as calving score increases from 1 to

3. This would be supportive of the increased stress associated with the increase in calving score. However, the lack of animals at a score of 3 reduces the importance that can be attached to the neutrophil:lymphocyte ratio at that score. In 1991, a similar pattern was seen.

Blood Hormones and Hematology in Calves at 24 Hours of Age.

At 24 hours of age, epinephrine levels were similar across all levels of calving score. However, in the 1991 calves (Table 13, page 65), epinephrine levels were elevated at a score of 1. This may have resulted from the handling of the calves while obtaining the 24 hour blood sample. Cortisol levels were lower than at birth in both years but a slight increase was still evident as the calving score increased from 1 to 3. Norepinephrine levels exhibited a similar pattern.

No distinct pattern was associated with T_3 levels at 24 hours. However, in the 1991 calves at 24 hours of age, T_3 levels were lower at calving scores of 2 and 3 as compared to a calving score of 1. This is interesting in that all calves were maintained inside the calving barn for the first 24 hours of life and it would be expected that these concentrations would be very similar. Perhaps, the stress associated with a more difficult parturition (a calving score of 2 or 3 as compared to 1) results in an impairment of T_3 secretion and thus the calves' ability to thermoregulate.

Gastrin levels at 24 hours did not exhibit any visible pattern. This is not unexpected as gastrin levels would be very heavily influenced by the suckling pattern of the calf during the time period previous to the drawing of the 24 hour blood sample.

Hemogram means were similar for the calves at 24 hours in both 1990 and 1991 (Tables 10 and 16, pages 63 and 69). However, a slight increase in percent eosinophils from birth to 24 hours of age was seen. This increase may have been indicative of the calves' recovery from the stress experienced at birth.

Regressions Utilizing Heifer Data.

Data from both 1990 and 1991 heifers were utilized to develop regression equations. A significant model was derived using the 1990 heifer data (Table 1, page 47) while the model based upon the 1991 heifer data had a statistically nonsignificant overall F-value. The error degrees of freedom value associated with the 1991 model was very low (d.f. = 6) due to missing data points. This may have impacted the significance level of the model.

In the 1990 model (Table 1, page 47 and Equation 1, page 40), significant interactions were seen between gastrin and epinephrine (Figure 1, page 48), and epinephrine and norepinephrine (Figure 2, page 49). In the gastrin by

epinephrine interaction (Figure 1, page 48) at a given level of gastrin, increases in epinephrine result in increases in calving score, to a maximum point. However, at lower levels of gastrin, calving score initially appears to decrease and then increase as gastrin levels increase.

In the epinephrine by norepinephrine interaction (Figure 2, page 49), as levels of norepinephrine increased, so did levels of epinephrine and resulted in increased calving scores. This interaction reached a maximum point, after which calving scores fell. The relationship between epinephrine and norepinephrine is expected as norepinephrine is converted to epinephrine by the enzyme PNMT. At lower levels of norepinephrine release, sufficient norepinephrine is available to be epinephrine secreting cells to be methylated to epinephrine.

The interaction is plotted over the mean values of the other explanatory variable utilized in the model. That is, the mean value of the other variable was used as a constant while the levels of epinephrine and norepinephrine changed over the range seen in the data set. This allowed visualization of the interaction. As the levels of the other variable changed, the shape of the interaction remains the same, it just moves up and down the y-axis (calving score). This was the method followed in plotting all of the interactions.

From this, it may be inferred that the magnitude of the effect of the interaction itself is small. That is, the interaction itself may be responsible for changes in calving score of a magnitude of only 1 or 2 units. This is also indicated by the regression coefficient on the interaction in the regression equation (0.0008--Equation 1, page 40).

This regression equation may be suspect due to the problems associated with the gastrin analysis, as described in the gastrin section.

Regression Utilizing Calf Data.

Regression equations were derived using both the 1990 and the 1991 calves at birth data.

In 1990 (Table 2, page 50 and Equation 2, page 42), interactions were seen between epinephrine and cortisol (Appendix Figure 2, page 101), norepinephrine and gastrin (Figure 5, page 53), norepinephrine and T_3 (Figure 4, page 52), cortisol and T_3 (Figure 3, page 51); and gastrin and T3 (Appendix Figure 3, page 102). Of these, the norepinephrine by gastrin, norepinephrine by T_3 and cortisol by T_3 interactions were significant.

The interaction of gastrin with norepinephrine (Figure 5, page 53) follows an expected pattern. As the levels of norepinephrine increase, gastrin levels decreased. Previous research has indicated that norepinephrine inhibits gastrin

and gastric acid secretion (19). These results also suggest that norepinephrine may exert an inhibitory effect on gastrin secretion.

The level of calving difficulty appears to increase with decreasing levels of T_3 and increasing levels of norepinephrine (Figure 4, page 52). Thompson, et al. (98) reported increasing levels of norepinephrine in response to cold stress. Increases in the level of T_3 are a typical response to cold stress. In this interaction, the level of calving difficulty or the duration of delivery may actually result in impaired T_3 secretion. This may explain why the interaction differs from what might be expected. This interaction may have also been impacted by the delay in the 1990 catecholamine analysis.

In examining the cortisol by T_3 interaction (Figure 3, page 51), the relationship is slightly different: calving scores are higher at higher levels of cortisol and T_3. In this interaction, cortisol appears to be increasing the levels of T_3 released. These results are similar to the response reported by Nathanielsz and Fisher (70).

In 1991, (Table 4, page 55 and Equation 4, page 44) interactions were seen between epinephrine and gastrin (Appendix Figure 4, page 103), cortisol and gastrin (Figure 7, page 57), and cortisol and T_3 (Figure 6, page 56). The

cortisol by gastrin and the cortisol by T_3 interactions were determined to be significant.

In the cortisol by gastrin interaction (Figure 7, page 57), as long as gastrin concentrations remained low, calving score was low over all levels of cortisol. This interaction, as plotted, may be misleading as there was only a very small number of observations with gastrin concentrations above 500 pg/ml. The effect of gastrin may actually be resulting from the levels of epinephrine. Although not statistically significant, an interaction was seen between epinephrine and gastrin (Appendix Figure 4, page 103) in this group of data. This interaction was supportive of results reported by Stadil (90).

The cortisol by T_3 interaction (Figure 6, page 56) shows that as levels of T_3 increase, calving scores increase over all levels of cortisol. However, when cortisol levels are low, the magnitude of the effect on calving score is increased. This relationship may indicate that if cortisol secretion is impaired, or reduced to exhaustion of available cortisol stores, the environmental temperature, as reflected by T_3 concentrations, may be more of a factor in calving stress.

The regression models developed utilizing each year's data resulted in a different equation with different interactions. The prevalence of T_3 interaction during the

1990 collection period may have resulted from the slightly lower temperatures during the 1990 calving season. Additionally, delays experienced in the gastrin and catecholamine analyses of the 1990 samples may make the 1991 regression model a more valid model.

Regression Utilizing Heifer and Calf Data Combined.

In 1990, a regression was developed relating heifer epinephrine with calf gastrin (Table 3, page 54 and Equation 3, page 43). Although the error degrees of freedom did not permit the analysis of any interactions, it was possible to examine main and quadratic effects of heifer and calf epinephrine and calf gastrin. From this model, it is evident that at higher calving scores heifer and calf epinephrine and calf gastrin are increasing. It may be possible that the release of calf gastrin is being impacted by the levels of maternal epinephrine.

Whether the levels of maternal epinephrine are crossing the placenta is not determined here. However, it is well established that the primary fetal catecholamine is norepinephrine (30, 51, 54) and it may be that the placental permeability barrier to epinephrine is not maintained over extended periods of time as may occur during dystocial deliveries (39).

It was not possible to evaluate a combined model using the 1991 calving data as a result of the low degrees of freedom associated with the 1991 heifers. Also, the calf gastrin levels may be misleading for the reasons previously identified. This may affect the validity of this model.

Effects of Calving Stress on Calves at 24 Hours of Age.

Attempts were made to develop a model linking the blood hormones at 24 hours to calving score using both the 1990 data and the 1991 data. The models that were determined to be best fit using Mallow's Cp[1] were not statistically significant models. The model developed using the 1990 data additionally had a very weak R^2 value.

Further, in examining Table 7, levels of epinephrine and cortisol appear to be similar at calving scores of 1, 2, and 4. In table 13, a similar trend is seen when comparing a calving score of 2 and 3.

It would appear from these results that 24 hour hormone levels are not an indicator of birth stress. By 24 hours of age, the calf appears to have adapted to its environment.

---

[1]Mallows, C.L. "Some Comments on C_p," Technometrics, 15, 661-675. 1973.

Conclusions

In this study it is very evident that parturition stress is a very complex event. This level of stress is impacted by many variables, i.e. temperature, environment, and level of dystocia. The calving scores assigned by the herdsmen in this project appear to be indicative of the level of stress experienced by the calf during parturition. Additionally, there appears to be a relationship between calf epinephrine levels and gastrin levels and a potential relationship between maternal epinephrine levels and the level of calf gastrin. Although not significant in this study, the interaction between epinephrine and gastrin in the calves at birth showed that at epinephrine levels increased over all concentrations of gastrin, there was an increase in calving score. However, this interaction resulted in the largest calving score at intermediate levels of gastrin (300 pg/ml) and calving score actually fell as gastrin levels increased.

Additionally, a potential relationship was seen between maternal and fetal epinephrine levels. However, the nature and the magnitude of this interaction needs to be further examined. It may also be of benefit to determine the relationship between neonatal epinephrine and gastrin concentrations and gastric pH.

Although not an objective of this study, a relationship between calving stress and low levels of T_3 was observed. At

higher calving scores, T_3 levels were observed to be lower than at lower calving scores and although the calves were maintained under similar environmental conditions, this discrepancy was still apparent at 24 hours of age. This relationship may have potential explanatory value regarding the survival of stressed calves and warrants futher investigation.

BIBLIOGRAPHY

1. Alvarez, M.B. and H.D. Johnson. 1970. Urinary excretion of adrenaline and noradrenaline in cattle during heat and cold exposure. J. Dairy Sci. 53:928-930.

2. Asteria, M.F. 1985. The physiology of stress. Human Sciences Press, Inc. New York.

3. Axelrod, J. and T.D. Reisine. 1984. Stress hormones: their interaction and regulation. Science. 224:452.

4. Banister, E.W. and J. Griffiths. 1972. Blood levels of adrenergic amines during exercise. J. Appl. Physiol. 33(5):674.

5. Bellows, R.A., R.B. Gibson, D.C. Anderson, and R.E. Short. 1971. Precalving body size and pelvic area relationships in hereford heifers. J. Anim. Sci. 33(2).

6. Bennington, J.L. Perinatal Pathology. 1984. W.B. Saunders Company. Philadelphia, Pennsylvania.

7. Blood, D.C., O.M. Radostits, and J.A. Henderson (Ed.). 1983. Diseases caused by physical and environmental influences. In: Veterinary Medicine (6th Ed.), Bailliere Tindall. London. p. 1239.

8. Breazile, J.E. 1988. The physiology of stress and its relationship to mechanisms of disease and therapeutics. In: Veterinary Clinics of North America-Food Animal Practice:Stress and Disease in Cattle. Vol. 4 No. 3.

9. Breazile, J.E., L.A. Bollmer, and L.E. Rice. 1988. Neonatal adaptation to stress of parturition and dystocia. In: Veterinary Clinics of North America-Food Animal Practice: Stress and Disease in Cattle. Vol. 4, No. 3.

10. Brinks, J.S., J.E. Olson, and E.J. Carroll. 1973. Calving difficulty and its association with subsequent productivity in herefords. J. Ani. Sci. 36(1):11.

11. Burfening, P.J. 1988. Relationship between age of dam with calving ease and birth weight of simmental calves. J. Anim. Sci. 66:841.

12. Cannon, W.B. 1929. Bodily Changes in Pain, Hunger, Fear, and Rage. Appleton, New York.

13. Cannon, W.B. 1929. Organization for physiological homeostasis. Physiol. Rev. 9(3):399.

14. Cannon, W.B. 1935. Stress and strains of homeostasis. Am. J. Med. Sci. 189(1):1.

15. Card, C.S., F.R. Spencer, E.H. Stauber, F.W. Frank, R.F. Hall, and A.C.S. Ward. 1973. The weak calf syndrome--epidemiology, pathology, and microorganisms recovered. U.S. Ani. Heal. Asso. Proc. 7:67.

16. Cheung, C.Y. 1984. Enhancement of adrenomedullary catecholamine release by adrenal cortex in fetus. Am. J. Physiol. 247(5 Part 1):E693.

17. Chin, A.K. and E. Evonuk. 1971. Changes in plasma catecholamine and corticosterone levels after muscular exercise. J. Appl. Physiol. 30(2):205.

18. Chin, A.K., R. Seaman, and M. Kapileshwarker. 1973. Plasma catecholamine response to exercise and cold adaptation. J. Appl. Physiol. 34:409.

19. Christensen, K.C. and F. Stadil. 1976. Effect of epinephrine and norepinephrine on gastrin release and gastric secretion of acid in man. Scand. J. Gastroent. Suppl. 7:87.

20. Cohen. W.R., G.J. Piasecki, and B.T. Jackson. 1982. Plasma catecholamines during hypoxemia in fetal lamb. Am. J. Physiol. 243(5):R520.

21. Comline, R.S., L.W. Hall, R.B. Lavelle, P.W. Nathanielsz, and M. Silver. 1974. Parturition in the cow: endocrine changes in animals with chronically implanted catheters in the fetal and maternal circulations. J. Endocrinol. 63:451.

22. Comline, R.S. and M. Silver. 1972. The composition of foetal and maternal blood during parturition in the ewe. J. Physiol. Lond. 178:211.

23. Comline, R.S. and M. Silver. 1966. Development of activity in the adrenal medulla of the foetus and newborn animal. Brit. Med. Bull. 22:16.

24. Davis. T.P., C.W. Gehrke, Jr., C.H. Williams, C.W. Gehrke, and K.O. Gerhardt. 1982. Pre-column derivatization and high-performance liquid chromatography of biogenic amines in blood of normal and malignant hyperthermic pigs. J. Chromotography. 228:113.

25. Dennis, S.M. Low viability of calves at birth. 1981. In: The Veterinary Annual. No. 21. G.S.G. Grunsell and F.W.G. Hill (Ed.). Bristol Scientechnica. Pg. 63.

26. Dierks, R.E. What is weak calf syndrome? 1976. Montana Nutr. Conf. Ann. Proc., 27th annual. 27:131.

27. Dimsdale, J.E. and J. Moss. 1980. Short-term catecholamines in stress and exercise. JAMA. 243(4):340.

28. Dixon, R., A. Hyman, E. Gurpide, I.Dyrenfurth, H. Cohen, E. Bowe, T. Engel, S. Daniel, S. James, and R. Vande Wiele. 1970. Feto-maternal transfer and production of cortisol in the sheep. Steroids. 16:771.

29. Eberhart, R.J. and J.A. Patt. 1971. Plasma cortisol concentrations in newborn calves. Am. J. Vet. Res. Vol. 32, No. 12:1921.

30. Eliot, R.J., A.H. Klein, R.H. Glatz, P.W. Nathanielsz, and D.A. Fisher. 1981. Plasma epinephrine, norepinephrine, and dopamine concentrations in maternal and fetal sheep during spontaneous parturition and in premature sheep during cortisol-induced parturition. Endocrinol. 08(5):1678.

31. Euler, U.S. von. 1964. Quantitation of stress by catecholamine analysis. Clin. Pharm. Therap. 5:398.

32. Farwell, S.O., R.A. Kagel, S.K. Gutenberger, and D.P. Olson. 1983. Weak calf syndrome and the determination of cortisol: adapting literature methods to real-life problems. Analytical Chemistry. 55(9):985A.

33. Faxelius, G. 1984. Sympathoadrenal activity and peripheral blood flow after birth: comparison in infants delivered vaginally and by C-section. J. Pediatr. 105:144.

34. Frankenhaeuser, M. 1975. Experimental approaches to the study of catecholamine and emotion. In: Emotions-their parameters and measurement. L. Levi (Ed.). Raven Press, New York.

35. Freeman, B.M. 1975. Aspects of stress in man and animals. Pro. Roy. Sco. Med. 68:427.

36. Godfry, R.W., S.D. Smith, M.J. Guthrie, R.L. Stanko, D.A. Neuendorff, and R.D. Randel. 1991. Physiological responses of newborn Bos indicus and Bos indicus X Bos taurus calves after exposure to cold. J. Anim. Sci. 69:258.

37. Graham, A.D.M., L.D. Longo, and C.Y. Cheung. 1986. Catecholamine secretion from the adrenal medulla of the fetus, regulation by hormones. J. of Developmental Physiology. 8:227.

38. Gray, I. and W.P. Beetham. 1967. Changes in plasma concentration of epinephrine and norepinephrine with muscular work. Proc. Soc. Exp. Biol. Med. 96:636.

39. Gu, Wei and C.T. Jones. 1986. The effect of elevation of maternal plasma catecholamines on the fetus and placenta of pregnant sheep. J. Develop. Physiol. 8(4):173.

40. Guyton, A.C. Textbook of Medical Physiology. 1981. W.B. Saunders Company. Philadelphia, Pennsylvania.

41. Hadley, M.E. 1988. Endocrinology. Prentice-Hall, Inc. Englewood Cliffs, New Jersey.

42. Hafez, E.S.E. 1987. Reproduction in farm animals. Lea and Febiger. Philadelphia, Pennsylvania.

43. Halliday, R. 1965. Failure of some hill lambs to absorb maternal gamma globulin. Nature (London). 205:614.

44. Harvey, S., J.G. Phillips, A. Rees, and T.R. Hall. 1984. Stress and adrenal function. J. Exp. Zoology. 232:633.

45. Hayes, J.R., J. Ardill, R.L. Kennedy, R.G. Shanks, and K.D, Buchanan. 1972. Stimulation of gastrin release by catecholamines. The Lancet. p. 819. April 15, 1972.

46. Holden, K.R., R.B. Yound, J.H. Piland, and W.G. Hurt. 1972. Plasma pressors in the normal and stress newborn infant. Pediatrics. 49:495.

47. Hostein, J., J. Fournet, J.L. Debru, and J. Bonnet-Eymard. 1978. Action of norepinephrine on the serum gastrin level. Digestion 18:134.

48. Houssay, B.A. and E.A. Molinelli. 1926. Adrenal secretion produced by asphyxia. Am. J. Physiol. 76(3):538.

49. Hunter, J.T., R.J. Fairclough, A.J. Peterson, and R.A.S. Welch. 1977. Fetal and maternal hormonal changes preceding normal bovine parturition. Acta. Endocrin. 84(3):653.

50. Ivanhoff, M.R. and H.W. Renshaw. 1975. Weak calf syndrome: Serum immunoglobulin concentrations in precolostral calves. Am. J. Vet. Res. 36(8):1129.

51. Izumi, F., R. Kushimoto, and M. Oka. 1980. Secretion of catecholamines in development. In: Biogenic Amines in Development. Parvez and Parvez (Ed.). Elsvier/North Holland. Biomedical Press. Amsterdam, New York, Oxford. p. 87.

52. Jain, N.C. (Ed.). 1986. Normal Hematology with comments on response to disease. In: Schalm's Veterinary Hematology (4th Ed.). Lea and Febiger. Philadelphia. p. 178.

53. Johnson, D.G., J.S. Hayward, T.P. Jacobs, M.L. Collis, J.D. Eckerson, and R.H. Williams. 1977. Plasma norepinephrine responses of man in cold water. J. Appl. Physiol. Respirat. Environ. Exercise Physiol. 48(2):216.

54. Jones C.R., J. McCullouch, L. Butlers, C.A. Hamilton, P.C. Rubin, and J.L. Reid. 1985. Plasma catecholamines and modes of delivery: the relations between catecholamine levels and in-vitro platelet aggregation and adrenoreceptor radioligand binding characteristics. Br. J. Obstet. Gynecol (England). 92(6):593.

55. Jones, C.T. 1980. Circulating catecholamines in the fetus, their origin, actions, and significance. In: Biogenic Amines in Development. Parvez and Parvez (Eds.) Elsvier/North Holland. Biomedical Press. Amsterdam, New York, Oxford. p. 63.

56. Jones, C.T., K. Boddy, J.S. Robinson, and J.G. Ratcliffe. 1972. Developmental changes in the responses of the adrenal glands of foetal sheep to endogenous adrenocorticotrophin, as indicated by hormone responses to hypoxaemia. J. Endo. 72:279.

57. Jones, C.T. and R.O. Robinson. 1975. Plasma catecholamines in foetal and adult sheep. J. Physio. (Lond.). 248:15.

58. Jones, C.T., R.O. Robinson, E. Luther, J.W.K. Ritchie, and D. Worthington. 1977. Control of adrenocorticotrophin secretion by catecholamines in the pregnant and foetal sheep. J. Endocr. 73:11.

59. Kasari, T.R. 1989. Weakness in neonatal calves associated with dystocia. Agri-Practice Reprod. 10(3):19.

60. Keller-Wood, M., J. Shinsako, and M.F. Dallman. 1984. Interaction between stimulus intensity and corticosteroid feedback in control of ACTH. Am. J. Physiol. 247:E489.

61. Keller-Wood, M., J. Shinsako, and M.F. Dallman. 1983. Integral as well as proportional adrenal responses to ACTH. Am. J. Physiol. 245:R53.

62. Keller-Wood, M. and M.F. Dallman. 1984. Corticosteroid inhibition of ACTH secretion. Endocri. Rev. 5:1.

63. Kotchen, T.A., L.H. Hartley, T.W. Rice, E.H. Mougey, L.G. Jones, and J.W. Mason. 1971. Renin, norepinephrine, and epinpehrine responses to graded exercise. J. Appl. Physiol. 31(2):178.

64. Lagercrantz, J. and P. Bistoletti. 1973. Catecholamine release in the newborn infant at birth. Pediatr. Res. 11:889.

65. Lagercrantz, J. and T.A. Slotkin. The "stress" of being born. Scientific American. p. 100, April 1986.

66. Laster, D.B., J.A. Glimp, L.V. Cundiff, and K.E. Gregory. 1973. Factors affecting dystocia and the effects of dystocia on subsequent reproduction in beef cattle. J. Ani. Sci. 36(4):695.

67. Martin, W.W. A complex disease. Agricultural Research. 24(9):3. 1974-1976.

68. Massip, A. 1980. The relation between the type of delivery and the acid-base and plasma cortisol levels of the newborn calf. Br. Vet. J. 136:488.

69. Morgan, C.D., M. Sandler, and M. Panigel. 1972. Placental transfer of catecholamines in vitro and in vivo. Am. J. Obstet. Gynecol. 112(8):1068.

70. Nathanielsz, P.W. and D.A. Fisher. 1979. Thyroid function in the perinatal period. Anim. Reprod. Sci. 2:57.

71. Padbury, J.F., J.K. Ludlow, M.G. Ervin, H.C. Jacobs, and J. A. Humme. 1987. Thresholds for physiological effects of plasma catecholamines in fetal sheep. Am. J. of Physiol. 252(4 Pt. 1):E 530.

72. Padbury, J.F., D.H. Polk, J.P. Newnham, and R.W. Lam. 1985. Neonatal adaptation: greater sympathoadrenal response in preterm than full-term fetal sheep at birth. Am. J. Physiol. 248(4):E443. 1985.

73. Perry, G. 1985. Aspects of stress in man and animals. Pro. Roy. Sco. Med. 68:423.

74. Pohorecky, L.A. and R.J. Wurtman. 1971. Adrenocortical control of epinephrine synthesis. Pharmacol. Rev. 23(1):1.

75. Rainforth, L.R. 1989. Maternal stress associated with neonatal disorders in calves. M.S. Thesis, University of Nebraska.

76. Randal G.C.B. 1978. Prenatal mortality: Some problems of adaptation at birth. In: Advances in Veterinary Science and Comparative Medicine. C. Cornelius (Ed.). London, Academic Press 22:53.

77. Richet, E., M.J. Davicco, and J.P. Barlet. 1985. Plasma catecholamine concentrations in lambs and calves during the perinatal period. Reprod. Nutr Develop. 25(6):1007.

78. Robertshaw, D. and G.C. Whittow. 1966. The effect of hyperthermia and localized heating of the anterior hypothalamus on the sympatho-adrenal system of the ox (Bos taurus). J. Physiol. 187:351.

79. Rohde, W., T. Ohkawa, K Dobashi, K. Arai, S. Okinaga, and G. Dorner. 1983. Acute effects of maternal stress on fetal blood catecholamines and hypothalamic LH-RH content. Clin. Endocrinol. 82(3):268.

80. Rosen K.G., L.H. Hokegard, and I. Kjellmer. 1975. A study of the relationship between the electrocardiogram and hemodynamics in the fetal lamb during asphyxia. Acta Physiol. Scand. 93:59.

81. Rubin, R.P. 1969. The metabolic requirements for catecholamine release from the adrenal medulla. J. Physiol. 202:197.

82. Selye, H.A. 1936. A syndrome produced by diverse nocuous agents. Nature. 138:32.

83. Selye, H. 1973. The evolution of the stress concept. Amer. Scientist. 61:692.

84. Shulkes, A., P. Chick, and K.J. Hardley. 1987. Nature of the gastric acid-gastrin feedback loop in the fetal sheep. Clin. Exp. Pharm. Phys. 14:503.

85. Silver, M. and A.V. Edwards. 1980. The development of the sympatho-adrenal system with an assessment of the role of the adrenal medulla in the fetus and neonate. In: Biogenic Amines in Development. Parvez and Parvez (Ed.). Elsvier/North Holland. Biomedical Press. Amsterdam, New York, Oxford. p. 147.

86. Silver, M., J.C. Ousey, F.E. Dudan, A.L. Foudan, J. Knox, R.S.S. Cash, and P.D. Rossdale. 1984. Studies on equine prematurity 2: postnatal adrenocortical activity in relation to plasma adrenocorticotrophic hormone and catecholamine levels in term and preterm foals. Equine Vet. J. 16(4):278.

87. Sippel, W.L. 1979. Weak calf syndrome. Cattlemen. 65:104.

88. Smyth, J.A., P.T. McNamee, D.G. Kennedy, S.J. McCullough, E.F. Logan, and W.A. Ellis. 1992. Stillbirth/perinatal weak calf syndrome: preliminary pathological, microbiological and biochemical findings. The Veterinary Record. March 21, 1992.

89. Sodeman, W.A., Jr. and T.A. Sodeman. 1985. W.B. Saunders Company. Philadelphia, Pennsylvania.

90. Stadil, F. and J.F. Rehfeld. 1973. Release of gastrin by epinephrine in man. Gastroentrology. 65(2):210.

91. Stauber, E.H. 1976. Weak calf syndrome: a continuing enigma. J. Vet. Med. Asso. 168(3):223.

92. Stephens, D. B. 1980. Stress and its measurement in domestic animals: a review of behavioral and physiological studies under field and laboratory situations. Adv. Vet. Sci. Comp. Med. 24:179.

93. Stott, G.H. 1980. What is animal stress and how is it measured? J. Ani. Sci. 52:150.

94. Stott, G.H. 1980. Immunoglobulin absorption in calf neonates with special consideration of stress. J. Dairy Sci. 63:681.

95. Stott, G.H. and E.J. Reinhard. 1978. Adrenal function and passive immunity in the dystocial calf. J. Dairy Sci. 61:1457.

96. Szenei O. and K. Nyiro. 1981. Assessment of the parameters controlling the acid-base status of newborn calves. Acta Vet. Acad. Sci. Hungaricae. 19:153.

97. Tennant, B., D.Harrold, M. Reina-Fuerra, J.W. Kendrick, and R.C. Laben. 1974. Hematology of the neonatal calf: erythrocyte and leukocyte values of normal calves. Cornell Vet. 64:516.

98. Thompson, J.R., R.J. Christopherson, V.A. Hammond, and G.A. Hills. 1978. Effects of actue cold exposure on plasma concentrations of noradrenaline and adrenaline in sheep. Can. J. Anim. Sce. 58:23.

99. Thompson, J.R., E.J. Pollack, and C.L. Pelissier. 1983. Interrelationships of parturition problems, production of subsequent lactation, reproduction and age at first calving. J. Dairy Sci. 66:1119.

100. Thorburn, G.D. and J.R.G. Challis. 1979. Endocrine control of parturition. Physiol. Rev. 59(4):863.

101. Thornburn, G.D., J.R.C. Challis, and W.B. Currie. 1977. Control of parturition in domestic animals. Biol. Reprod. 16:18.

102. Thorburn, G.D., D.H. Nicol, J.M. Bassett, D.A. Shutt, and R.I. Cox. 1972. Parturition in the goat and sheep: changes in corticosteroids, progesterone, oestrogens and prostaglandin. F. J. Reprod. Fert. 16:(suppl.):35.

103. Trifaro, J.M. 1970. The secretory process of the adrenal medulla (review). Endocrinol. Exp. 4:225.

104. Walsh, J.H. and M.I. Grossman. 1975. Gastrin. New Eng. J. of Med. 292(25):1324.

105. Ward, J.K. 1981. Weak calf syndrome. In: Current Veterinary Therapy: Food Animal Practice. J.L. Howard (Ed.). p. 105.

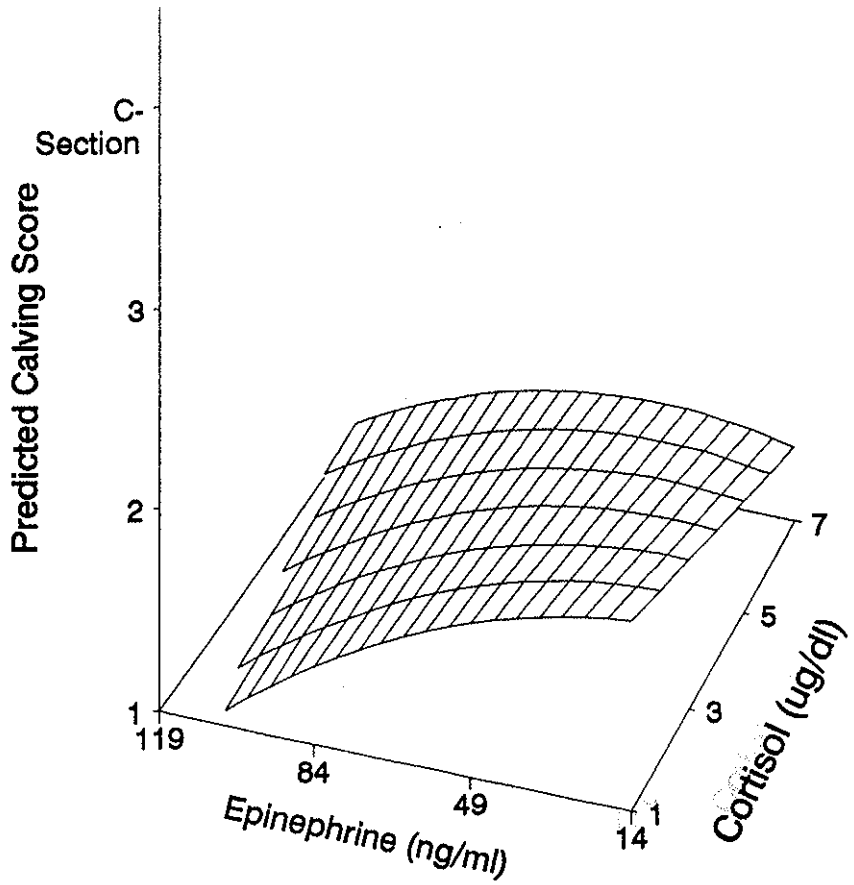
106. Weisbrot, I.M., L.S. James, C.E. Prince, D.A. Holaday, and B. Apgar. 1958. Journal of Pediatrics. 52:395.

107. Woodward, R.R. and R.T. Clark. A study of stillbirths in a herd of range cattle. 1959. J. Ani. Sci. 18:85.

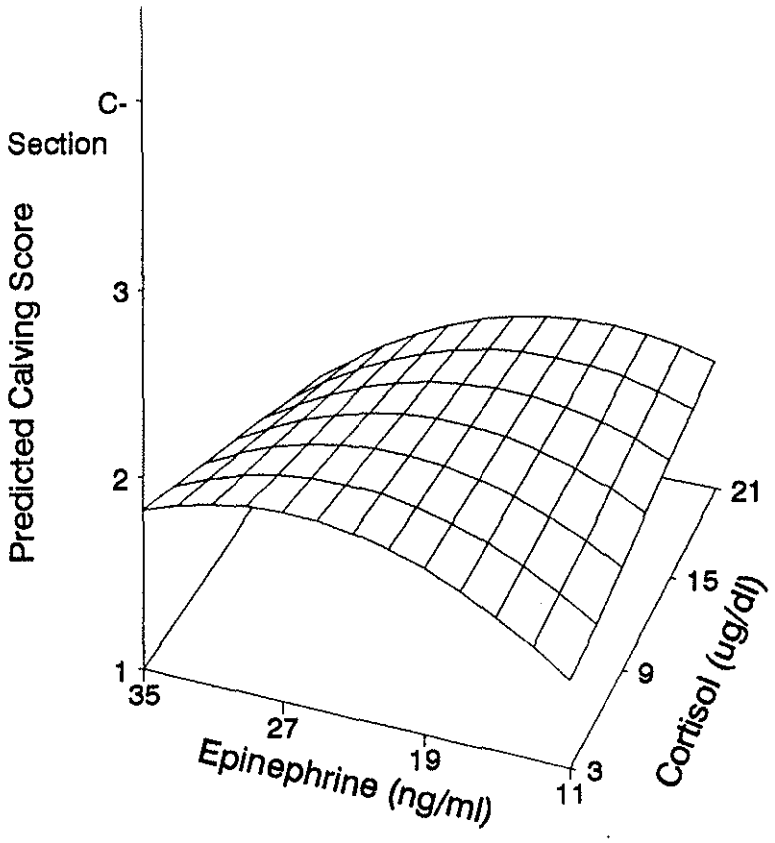
108. Yasuda, K., K. Ono, A. Hasegawa, and I. Tomoda. 1986. Plasma gastrin levels in cows at various growing stages. Jpn. J. Vet. Sci. 48(6):1109.

APPENDIX

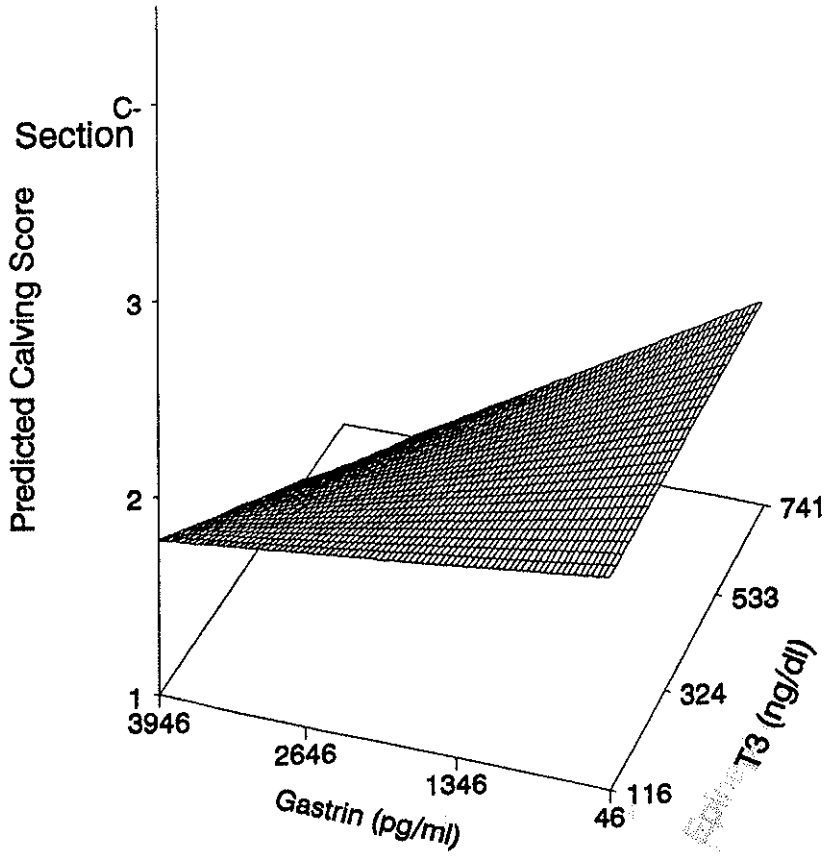
Appendix Figure 1: Epinephrine X Cortisol Interaction for 1990 Heifers at Parturition



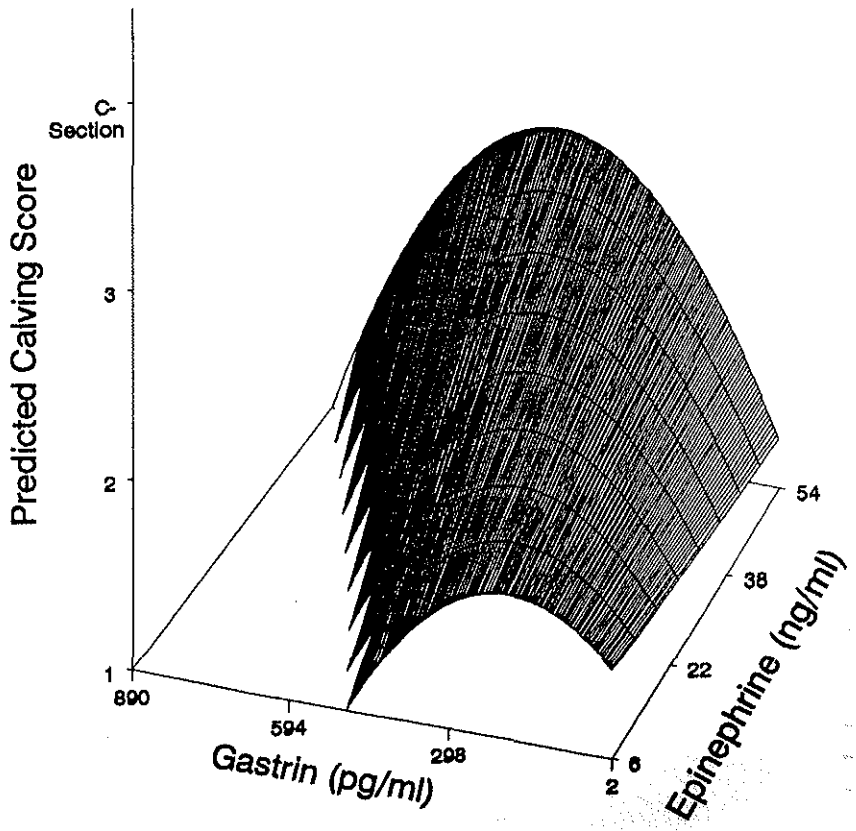
Appendix Figure 2: Epinephrine X Cortisol Interaction for 1990 Calves at Parturition



Appendix Figure 3: Gastrin X
T3 Interaction for 1990
Calves at Parturition



Appendix Figure 4: Gastrin X Epinephrine Interaction for 1991 Calves at Parturition



Daily temperatures, windspeed, approximate windchill temperature during the 1990 sample period.

DATE	HIGH TEMP(F)	LOW TEMP(F)	AVERAGE WIND(MPH)	AVERAGE WINDCHILL
3-1	55.56	14.31	7.48	22.0
3-2	-	-	-	-
3-3	51.26	15.21	-	-
3-4	63.97	25.90	7.29	38.5
3-5	55.27	25.90	10.89	28.0
3-6	36.80	33.49	16.32	16.0
3-7	41.26	34.38	14.31	16.0
3-8	50.09	32.24	8.02	28.0
3-9	-	-	-	-
3-10	68.52	39.86	-	-
3-11	63.73	53.87	16.93	-
3-12	70.90	46.89	6.96	-
3-13	66.15	39.44	-	-
3-14	47.52	34.66	-	-
3-15	49.95	29.50	-	-
3-16	51.69	27.70	-	-
3-17	-	-	-	-
3-18	51.40	18.27	-	-
3-19	51.40	18.27	-	-
3-20	68.68	29.16	-	-
3-21	73.69	35.90	-	-
3-22	52.27	27.97	-	-
3-23	27.97	16.90	-	-
3-24	31.69	15.13	-	-
3-25	48.24	12.56	-	-
3-26	52.34	24.15	-	-
3-27	54.09	27.77	-	-
3-28	42.16	34.18	-	-
3-29	38.68	32.45	-	-
3-30	-	-	-	-
3-31	57.81	37.78	-	-
4-1	55.42	30.31	-	-
4-2	56.61	25.75	-	-
4-3	66.70	26.94	-	-
4-4	61.86	38.96	-	-
4-5	49.03	19.56	-	-
4-6	52.05	19.13	-	-
4-7	66.06	21.97	-	-
4-8	69.28	32.66	-	-
4-9	59.94	44.20	-	-
4-10	51.40	24.01	13.37	22.0

1990 Daily temperatures continued:

DATE	HIGH TEMP(F)	LOW TEMP(F)	AVERAGE WIND (MPH)	AVERAGE WINDCHILL
4-11	48.02	17.19	8.00	21.5
4-12	42.02	20.77	8.54	22.0
4-13	60.85	33.35	6.78	-
4-14	71.24	25.48	8.40	-
4-15	65.50	40.49	7.15	-
4-16	66.22	37.22	11.31	-
4-17	52.34	22.60	6.23	35.0
4-18	54.75	40.42	16.04	-
4-19	58.71	42.93	13.83	-
4-20	74.95	43.64	4.87	-
4-21	78.69	41.53	6.18	-
4-22	88.95	52.63	13.19	-
4-23	83.50	62.01	15.53	-
4-24	80.55	57.20	16.78	-
4-25	72.99	57.43	15.66	-
4-26	74.05	52.99	11.57	-
4-27	-	-	-	-
4-28	-	-	-	-
4-29	55.49	33.35	-	-
4-30	62.94	30.11	7.12	-
5-1	63.73	29.77	5.47	-
5-2	63.90	43.99	6.81	-
5-3	53.29	42.93	14.08	-
5-4	-	-	-	-
5-5	66.06	42.79	0.15	-

Daily temperatures, windspeed, approximate windchill temperature during the 1991 sample period.

DATE	HIGH TEMP(F)	LOW TEMP(F)	AVERAGE WIND(MPH)	AVERAGE WINDCHILL
3-10	71.06	26.17	11.05	-
3-11	71.15	30.45	6.52	-
3-12	47.88	31.63	16.85	22.0
3-13	31.76	24.85	13.87	5.5
3-14	39.93	15.80	6.26	22.0
3-15	34.94	22.68	6.65	23.3
3-16	39.93	31.35	8.10	22.0
3-17	45.10	31.35	10.60	27.0
3-18	67.86	26.94	8.97	-
3-19	61.00	26.94	9.05	35.5
3-20	67.05	39.51	13.91	-
3-21	69.96	30.25	5.61	-
3-22	47.16	36.18	11.73	28.0
3-23	60.85	32.38	15.59	29.0
3-24	74.77	28.80	8.02	-
3-25	77.07	37.15	9.99	-
3-26	78.98	41.82	9.86	-
3-27	51.48	24.78	16.51	22.0
3-28	56.39	26.94	8.66	32.5
3-29	37.78	23.59	7.22	27.0
3-30	57.29	19.99	9.45	19.0
3-31	68.20	31.42	7.85	-
4-1	75.96	34.04	7.36	-
4-2	63.57	41.75	11.03	-
4-3	64.69	43.01	4.95	-
4-4	77.18	36.53	5.84	-
4-5	86.94	46.53	-	-
4-6	86.59	54.39	15.99	-
4-7	83.50	54.75	12.85	-
4-8	68.94	42.09	12.44	-
4-9	59.47	33.21	10.54	-
4-10	62.33	48.60	16.28	-
4-11	56.68	41.12	19.85	-
4-12	70.12	41.82	14.65	-
4-13	53.94	38.33	11.61	-
4-14	57.96	32.31	5.90	-
4-15	70.81	38.61	6.41	-
4-16	56.84	37.79	12.52	-
4-17	58.87	42.23	10.90	-
4-18	52.20	44.76	8.82	-
4-19	45.54	41.33	9.01	34.0
4-20	58.95	36.74	4.95	-
4-21	48.96	41.26	5.66	43.0
4-22	65.08	38.54	8.39	-
4-23	62.56	37.98	7.71	-

1991 Daily temperatures continued:

DATE	HIGH TEMP (F)	LOW TEMP(F)	AVERAGE WIND (MPH)	AVERAGE WINDCHILL
4-24	65.25	32.66	9.16	-
4-25	66.54	43.50	13.54	-